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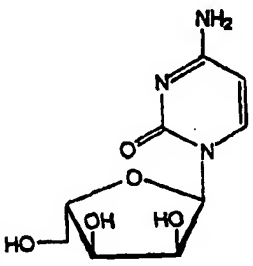
(54) Title: OLIGONUCLEOTIDES MODIFIED WITH NOVEL α-L-RNA ANALOGUES



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(57) Abstract: The invention relates to novel α-L-RNA monomers, which, when incorporated into an oligonucleotide impair a higher tendency towards hybridization with a RNA complement, as comparred to a DNA complement. The invention also relates to a process for the preparation of an α-L-RNA modified oligonucleotide and an intermediate for manufacturing the same. The novel oligonucleotides are useful for a variety of therapeautic, diagnostic, and general molecular biology applications.

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OLIGONUCLEOTIDES MODIFIED WITH NOVEL α-L-RNA ANALOGUES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the field of providing oligonucleotides modified with one or more α-L-configurated nucleoside analogues. Further, the invention relates to synthetic oligonucleotides capable of forming nucleobase-specific duplexes with complementary single stranded nucleic acids, and to the combination of α-L-RNA and LNA (Locked Nucleic Acid) nucleotides in the formation of said oligonucleotides. The invention also relates to the use of oligonucleotides comprising α-L-configurated nucleosides and nucleic acid derivatives comprising the same as therapeutic drugs.

2. Summary

Synthetic oligonucleotides are widely used compounds in disparate fields, such as molecular biology, DNA/RNA-based diagnostics, and therapeutics. To be useful for a range different applications, oligonucleotides have to satisfy a large number of different requirements. As therapeutics, for instance, a useful oligonucleotide must be able to penetrate the cell membrane, have good resistance to extra- and intracellular nucleases, and desirably have the ability to recruit endogenous enzymes like RNase H. In DNA-based diagnostics and molecular biology other properties are important. For instance, the ability of oligonucleotides to act as efficient substrates for a wide range of different enzymes evolved to act on natural nucleic acids, such as polymerases, kinases, ligases and phosphatases maybe desirable.

A fundamental property of oligonucleotides is their ability to recognize and hybridize sequence-specifically to complementary single stranded nucleic acids ("to pair"), employing either Watson-Crick hydrogen bonding (A-T and G-C) or other hydrogen bonding schemes, such as the Hoogsteen mode. There are two important terms, affinity and specificity, commonly used to characterize the hybridization properties of a given oligonucleotide. Affinity is a measure of the binding strength of the oligonucleotide to its complementary target sequence (expressed as the thermostability (T_m) of the duplex). Each nucleobase that is paired adds to the thermostability of the duplex. Thus, affinity increases with increasing size (number of nucleobases) of the oligonucleotide. Specificity is a measure of the ability of the oligonucleotide to discriminate between a fully complementary and a mismatched target sequence. In other words, specificity is a measure of the loss of affinity associated with mismatched nucleobase pairs in the target. The term "specificity" may, in the present context, also be used to describe the ability of an oligonucleotide to discriminate between RNA and DNA based sequences.

At constant oligonucleotide size, the specificity increases with increasing number of mismatches between the oligonucleotide and its targets (i.e., the percentage of mismatches increases). Conversely, specificity decreases when the size of the oligonucleotide is increased at a constant number of mismatches (i.e., the percentage of mismatches decreases). Stated another way, an increase in the affinity of an oligonucleotide occurs at the expense of specificity and vice-versa. Given the shortcomings of natural oligonucleotides, new approaches for enhancing specificity and affinity are highly desirable for RNA/DNA-based therapeutics, diagnostics, and for molecular biology techniques in general.

It is known that oligonucleotides undergo a conformational transition in the course of hybridizing to a target sequence, from the relatively random coil structure of the single stranded state to the ordered structure of the duplex state.

Thus, conformational restriction has recently been applied to oligonucleotides in the search for analogues displaying improved hybridization properties compared to the unmodified (2'-deoxy) oligonucleotides.

Locked Nucleic Acids (LNAs) are an example of conformationally restricted monomers. LNAs are reported in WO 99/14226 and in various scientific publications, including Nielsen, P., Pfundheller, H. M., Olsen, C. E. and Wengel, J., *J. Chem. Soc., Perkin Trans. 1,* **1997**, 3423; Nielsen, P., Pfundheller, H. M., Wengel, J., *Chem. Commun.,* **1997**, 9,825; Christensen, N. K., Petersen, M., Nielsen, P., Jacobsen, J. P. and Wengel, J., *J. Am. Chem. Soc.,* **1998**, *120*, 5458; Koshkin, A. A. and Wengel, J., *J. Org. Chem.,* **1998**, *63*, 2778; Obika, S., Morio, K.-I., Hari, Y. and Imanishi, T., *Bioorg. Med. Chem. Lett.*, **1999**, 515. Interestingly, incorporation of LNA monomers containing a 2'-O,4'-C-methylene bridge (β-D-LNA) into an oligonucleotide sequence led to unprecedented improvement in the hybridization ability of the modified oligonucleotide (Singh, S. K., Nielsen, P., Koshkin, A. A., Olsen, C. E. and Wengel, J., *Chem. Commun.*, **1998**, 455; Koshkin, A. K., Singh, S. K., Nielsen, P., Rajwanshi, V. K., Kumar, R., Meldgaard, M., Olsen, C. E., and Wengel, J., *Tetrahedron*, **1998**, 54, 3607; Koshkin, A. A. Rajwanshi, V. K., and Wengel, J., *Tetrahedron Lett.*, **1998**,*39*,4381; Singh, Sanjay K. and Wengel, J., *Chem. Commun.*, **1998**, 1247; Kumar, R., Singh, S. K., Koshkin, A. A., Rajwanshi, V. K., Meldgaard, M., and Wengel, J., *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2219; Obika, S. et al. *Tetrahedron Lett.*, **1997**, 38, 8735; Obika, S. et al. *Tetrahedron Lett.*, **1998**, 39, 5401; Singh, S. K., Kumar, R., and Wengel, J., J. *Org. Chem.*, **1998**, 63, 6078; Koshkin, A. A., Nielsen, P., Meldgaard, M., Rajwanski, V. K., Singh, S. K., and Wengel, J., *J. Am. Chem. Soc.*, **1998**, *120*, 13252; Singh, S. K., Kumar, R., and Wengel, J., *J. Org. Chem.*, **1998**, 63, 10035).

Oligonucleotides comprising these LNA monomers and the corresponding 2'-thio-LNA analogues form duplexes with complementary DNA and RNA with

thermal stabilities not previously observed for bi- or tricyclic nucleosides modified oligonucleotides (T_m change/modification = +3°C to +11°C). These oligonucleotides also show improved selectivity.

Variations of the stereochemistry on the furanosyl group of LNA have been studied. In WO 00/56748, the xylo-analogues of LNA are reported to have an increased affinity compared to LNA, at least when a plurality of *xylo*-LNAs are placed in succession. In WO 00/66604, α-L-LNA analogues are demonstrated to have an increased affinity towards a complementary strand when incorporated into an oligonucleotide.

Antisense oligonucleotides are useful as therapeutic agents. Briefly, an antisense drug operates by binding to the mRNA, thereby blocking or modulating its translation into protein. Thus, antisense drugs may be used to directly block the synthesis of disease causing proteins. They may, of course, also be used to block synthesis of normal proteins in cases where these participate in, and aggravate, a pathophysiological process. It should be emphasized that antisense drugs can also be used to activate genes rather than suppressing them by, for example, blocking the synthesis of a protein that otherwise suppresses RNA synthesis from the target gene. Mechanistically, the hybridizing oligonucleotide is thought to elicit its effect by either creating a physical block to the translation process or by recruiting a cellular enzyme (RNase H) that specifically degrades the mRNA part of the mRNA/antisense oligonucleotide duplex. Hence, nucleic acids that are antisense to the transcript of a specific gene, such as a gene key to a pathogen or a deleterious human gene, such as those involved in certain cancers, could impair the expression of this gene, thereby disabling the particular disease state.

The main advantages of the antisense approach to therapy are specificity and point of attack. Antisense targets can be selected that are unique to the gene whose expression is to be controlled. Hence, only that gene's expression is inhibited. This is especially important for diseases like viral infections and

cancers which employ normal cell functions as part of the disease process. It has been difficult to devise therapeutic strategies against these diseases without also disabling normal cell functioning. Antisense nucleic acids targeted toward a gene that is diverting these normal cell functions, however, can specifically impair the disease state without affecting cell function. An antisense approach, therefore, has the promise of fewer side effects and offers real therapeutic promise for certain cancers and viral diseases.

Another advantage to an antisense approach is that it inhibits the pathogenic process at the source of the disease. That is, it interferes with the formation of unwanted proteins, rather than stopping these proteins from functioning. In addition, technically it is easier to identify nucleic acid targets than it is to identify protein targets.

Not unexpectedly, oligonucleotides must satisfy a large number of different requirements to be useful as antisense drugs. Ideally, among the desirable properties of antisense oligonucleotide are binding with high affinity and specificity to its target mRNA, the ability to recruit RNase H, the ability to reach their site of action within the cell, resistant and to extra- and intracellular nucleases (both endo- and exo-nucleases), little or not toxicity at the relevant dose, and the ability to specifically hybridize to mRNA, leaving DNA unhybridized at physiological conditions.

Natural DNA only exhibits modest affinity for RNA and falls short on a number of the other critical characteristics, especially nuclease resistance. Hence, a significant effort has been invested to identify novel analogues with improved antisense properties. In particular, the search has focused on identifying novel analogues that combine an increased affinity for complementary nucleic acids with the RNase H recruiting ability of natural DNA. Both of these properties have been demonstrated to correlate positively with the antisense molecule's activity. In WO 01/25248, a promising method is

disclosed in which bicyclic DNA analogues are incorporated into an oligonucleotide which recruits RNase H when hybridized to an RNA target sequence.

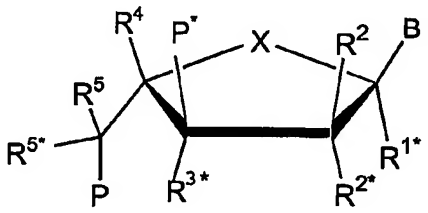
Normally, oligonucleotides hybridize to RNA as well as DNA with comparative affinity, with an affinity slightly biased toward either the complementary RNA or the DNA. There is a risk that an antisense oligonucleotide directed towards a target mRNA may unintentionally hybridize to genomic DNA, optionally via strand displacement, and cause toxic side effects. Thus, there is a need for antisense oligonucleotides that have greater affinity for RNA (e.g., mRNA) than DNA (e.g., genomic DNA). An oligonucleotide selective towards RNA is also desirable for preparative purification of RNA from mixtures comprising DNA and RNA.

SUMMARY OF THE INVENTION

Improved Nucleic Acids and Methods for Producing Them

In one aspect of the present invention, the object is to provide an oligonucleotide that selectively hybridizes to RNA. An oligonucleotide showing this property may find various uses, including therapeutic and diagnostic utitlities. A further object according to the present invention is to avoid or reduce toxic side effects of antisense or other gene-silencing oligonucleotides (e.g., double-stranded nucleic acids) by reducing or eliminating their affinity towards DNA. A still further object of the present invention is to make available oligonucleotides which, in a single step, can selectively extract RNA from a sample containing a mixture of biological substances.

In another aspect, the invention provides an oligonucleotide (hereinafter termed "α-L-RNA modified oligonucleotide") comprising at least one oligonucleotide analogue (hereinafter termed "α-L-RNA monomer") of the general formula:



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where X is selected from -O-, -S-, -S(O)-, -$S(O)_2$-, -$N(R^{N*})$-, -$C(R^6R^{6*})$-, -O-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-O-, -S-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-S-, -$N(R^{N*})$-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-$N(R^{N*})$-, and -$C(R^6R^{6*})$-$C(R^7R^{7*})$-; B is selected from hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, optionally protected nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; P designates a radical position for an internucleoside linkage to a preceeding or successive monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R.^{5} , P* designates an internucleoside linkage to a preceding or successive monomer, or a 3'-terminal group. R^2 represents F, Cl, Br, I, SR", SeH, SeR", $N(R^{N*})_2$, OH, a protected hydroxy group, SH, a protected mercapto group, an optionally substituted linear or branched C_{1-12}-alkoxy, an optionally substituted linear or branched C_{2-12}-alkenyloxy. Each of the substituents R^{1*}, R^{2*}, R^{3*}, R^4, R^5, R^{5*}, R^6, R^{6*}, R^7, and R^{7*} is independently selected from hydrogen, optionally substituted linear or branched C_{1-12}-alkyl, optionally substituted linear or branched C_{2-12}-alkenyl, optionally substituted linear or branched C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-

carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, hetero-aryloxy, hydroxy protection group, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkylaminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkylcarbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, azido, sulfanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond. R", when present, represents a C_{1-6}-alkyl or phenyl group. R^{N*}, when present, is selected from hydrogen and C_{1-4}-alkyl. Oligonucleotides containing at least one monomer of the formula (I) may also comprise basic salts and acid addition salts thereof.

The nucleobase B may be selected from a variety of substituents. In one embodiment, B designates a naturally-occuring nucleobase selected from uracil-1-yl, thymin-1-yl, adenin-9-yl, guanin-9-yl, cytosin-1-yl, and 5-methylcytosin-1-yl. In other embodiments,, B is a non-naturally-occurring nucleobase.

The oligonucleotide according to the invention desirably contains at least one α-L-RNA monomer, wherein X is selected from the group consisting of -O-, -S-, -S(O)-, $-S(O)_2-$, and -($N(R^{N*})$-. Desirably, X represents -O-.

The substituents R^{1*}, R^{2*}, R^{3*}, R^4, R^5, and R^{5*} may, in a desired embodiment independently represent hydrogen, C_{1-4}-alkyl, or C_{1-4}-alkoxy. In one embodiment, R^2 represents hydrogen. In another embodiment, R^2 represents a protected hydroxy group.

An exemplary hydroxy protecting group for R^2 may be a linear or branched C_{1-6}-alkoxyl group or a silyloxy group substituted with one or more linear or branched C_{1-6}-alkyl groups. Desirably, the substituent R^2 is tert-butyldimethylsilyloxy.

The substituent P, when representing a 5'-terminal group, suitably designates hydrogen, hydroxy, optionally substituted linear or branched C_{1-6}-alkyl, optionally substituted linear or branched C_{1-6}-alkoxy, optionally substituted linear or branched C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, monophosphate, diphosphate, triphosphate, or -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands. Desirably, when it is a 5'-terminal group, P represents hydroxy or dimethoxytrityloxy.

The oligonucleotides according to any one of the previously described analogs, wherein P*, when representing a 3'-terminal group, suitably is selected from hydrogen, hydroxy, optionally substituted linear or branched C_{1-6}-alkoxy, optionally substituted linear or branched C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}- alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

When P and P^* represent an internucleoside linkage between a successive and a preceding monomer, P and P^* independently suitably may be selected from the group consisting of from -CH_2-CH_2-CH_2-, -CH_2-CO-CH_2-,

$-CH_2-CHOH-CH_2-$, $-O-CH_2-O-$, $-O-CH_2-CH_2-$, $-O-CH_2-CH=$, $-CH_2-CH_2-O-$, $-NR^H-CH_2-CH_2-$, $-CH_2-CH_2-NR^H-$, $-CH_2-NR^H-CH_2-$, $-O-CH_2-CH_2-NR^H-$, $-NR^H-CO-O-$, $-NR^H-CO-NR^H-$, $-NR^H-CS-NR^H-$, $-NR^H-C(=NR^H)-NR^H-$, $-NR^H-CO-CH_2-NR^H-$, $-O-CO-O-$, $-O-CO-CH_2-O-$, $-O-CH_2-CO-O-$, $-CH_2-CO-NR^H-$, $-O-CO-NR^H-$, $-NR^H-CO-CH_2-$, $-O-CH_2-CO-NR^H-$, $-O-CH_2-CH_2-NR^H-$, $-CH=N-O-$, $-CH_2-(C=NR^H)-O-$, $-CH_2-O-N=$, $-CH_2-O-NR^H-$, $-CO-NR^H-CH_2-$, $-CH_2-NR^H-O-$, $-CH_2-NR^H-CO-$, $-O-NR^H-CH_2-$, $-O-NR^H-$, $-O-CH_2-S-$, $-S-CH_2-O-$, $-CH_2-CH_2-S-$, $-O-CH_2-CH_2-S-$, $-S-CH_2-CH=$, $-S-CH_2-CH_2-$, $-S-CH_2-CH_2-O-$, $-O-CH_2-CH_2-O-$, $-S-CH_2-CH_2-S-$, $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, $-CH_2SO_2-CH_2-$, $-O-SO-O-$, $-O-S(O)_2-O-$, $-O-S(O)_2-CH_2-$, $-O-S(O)_2-NR^H-$, $-NR^H-S(O)_2-CH_2-$, $-O-S(O)_2-CH_2-$, $-O-P(O)_2-O-$, $-O-P(O,S)-O-$, $-O-P(S)_2-O-$, $-S-P(O)_2-O-$, $-S-P(O,S)-O-$, $-S-P(S)_2-O-$, $-O-P(O)_2-S-$, $-O-P(O,S)-S-$, $-O-P(S)_2-S-$, $-S-P(O)_2-S-$, $-S-P(O,S)-S-$, $-S-P(S)_2-S-$, $-O-PO(R")-O-$, $-O-PO(OCH_3)-O-$, $-O-PO(BH_3)-O-$, $-O-PO(NHR^N)-O-$, $-O-P(O)_2-NR^H-$, $NR^H-P(O)_2-O-$, $-O-P(O,NR^H)-O-$, and $O-Si(R")_2-O-$, wherein R^H represent hydrogen or a linear or branched C_{1-4}-alkyl group, and R" represent a C_{1-6}-alkyl or phenyl group. Desirably, any internucleoside linkage independently is selected from $-CH_2-CO-NR^H-$, $-CH_2-NR^H-O-$, $-S-CH_2-O-$, $-O-P(O)_2-O-$, $-O-P(O,S)-O-$, $-O-P(S)_2-O-$, $-NR^H-P(O)_2-O-$, $-O-P(O,NR^H)-O-$, $-O-PO(R")-O-$, $-O-PO(CH_3)-O-$, and $-OPO(NHR^H)-O-$. Most desirable as internucleoside linkage is $-O-P(O)_2-O-$, which is the natural occuring linkage.

The present invention also provides an oligonucleotide comprising at least one α-L-LNA monomer and at least one α-L-RNA monomer. Surprisingly, oligonucleotides with this composition express an enhanced ability to hybridize selectively to RNA.

In one embodiment, at least one α-L-LNA monomer and at least one α-L-RNA monomer are positioned relative to each other in any order. As an example, at least one α-L-LNA monomer may be positioned in the oligonucleotide upstream or downstream relative to at least one α-L-RNA monomer. Usually, it is desirable that at least one α-L-LNA monomer is placed adjacent to at least one α-L-RNA monomer in the oligonucleotide.

In one aspect of the invention, the oligonucleotide comprises a group or groups of at least one α-L-LNA monomer alternating with group(s) of at least one α-L-RNA, optionally with intervening natural occurring or synthetic nucleosides. Each of the groups of α-L-LNA and α-L-RNA desirably comprise 1 to 10, more desirably, 2 to 8 nucleosides. The alternating groups may be present in a block placed at the 3' or 5' end of the oligonucleotide, or the block may be situated in the central part of the oligonucleotide flanked by naturally occurring or synthetic nucleosides. More than one block may be present in the oligonucleotide. When alterations occur in the oligonucleotide, typically 1 to 50 alternations, 1 to 25 alterations, 1 to 15 alterations, or 2 to 10 alterations are present.

The oligonucleotide may have any suitable length. The oligonucleotide may have, e.g., 1-100 α-L-LNA monomers, 1-100 α-L-RNA monomers, and 0-1000 nucleosides selected from naturally-occurring or synthetic nucleosides. Desireably, the total number of nucleotide monomer does not exceed 1000, and is not less than 3. In a desired embodiment, the number of nucleotide monomers is not above 100, and is not less than 8. Desirably, the total number of nucleotide monomers is at least 12. Most desirably, the total number of nucleotide monomers is 2 to 8. In other embodiments, the oligonucleotide is between 1000 to 2000, 2001 to 3000, 3001 to 5000, or 5001 to 10,000 nucelotides in length, inclusive. However, the length of the nucleotide can be readily adjusted by one skilled in the art depending on the intended application

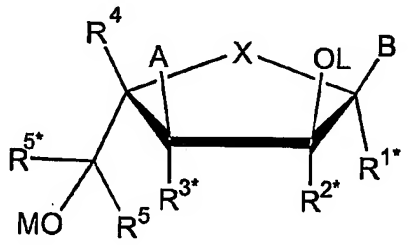
of the oligonucleotide. As an example, when used as a probe in SNP detection, a suitable length of the nucleotides may be, e.g., 12 to 25 nucleoside monomers, while the length of the nucleotide in the study of expression of alternative splicing may be, e.g., between 50 and 100 nucleoside monomers.

The oligonucleotide comprises in a desired embodiment at least 3 α-L-LNA monomers and/or at least 3 α-L-RNA monomers. As indicated above, the α-L-LNA and α-L-RNA monomers may optionally be present as groups of two or more continuous α-L-LNA or α-L-RNA monomers. Alternatively, the oligonucleotide comprises a sequence wherein α-L-LNA monomers alternate with α-L-RNA monomers.

The synthetic nucleotide monomer, which optionally may be present in the oligonucleotide, may in one embodiment of the invention be β-D-LNA. In a desired embodiment, the synthetic nucleotide monomer is β-D-oxy-LNA. To increase the specificity it may be desirable to incorporate a contiguous stretch of 2 to 30 β-oxy-LNA monomers in the oligonucleotide of the invention. In a desired embodiment, a contiguous stretch of 2 to 30 β-oxy-LNA monomers in the oligonucleotide of the invention comprises at least one thymin-y-yl. In another desirable embodiment, a contiguous stretch of 2 to 30 β-oxy-LNA monomers having at least one thymin-y-yl is exclusively of the α-L-RNA configuration.

For some applications it may be advantageous to complex or to non-covalently or covalently bind an oligonucleotide of the invention to a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, DNA, RNA, and peptide nucleic acids (PNAs). For use in assays, such as microarrays or ELISA capture assays, usually, the oligonucleotide of the invention is complexed with a suitable linker to obtain a better performance.

In another aspect, the invention features a compound (e.g., an intermediate compound useful in the synthesis of an oligonucleotide of the invention) having the general formula II



II

where X is selected from -O-, -S-, -S(O)-, $-S(O)_2-$ $-N(R^{N*})-$, $-C(R^6R^{6*})-$, $-O-C(R^7R^{7*})-$, $-C(R^6R^{6*})-O-$, $-S-C(R^7R^{7*})-$, $-C(R^6R^{6*})-S-$,$-N(R^{N*})-C(R^7R^{7*})-$, $-C(R^6R^{6*})-N(R^{N*})-$, and $-C(R^6R^{6*})-C(R^7R^{7*})-$. R^{N*}, when present, is selected from hydrogen and C_{1-4}-alkyl; B is selected from hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, optionally protected nucleobases; DNA intercalators, photochemically active groups, thermo-chemically active groups, chelating groups, reporter groups, and ligands. L represents hydrogen or a hydroxy protection group; and M represents hydrogen or a hydroxy protection group. A represents a phosphoramidite group of the general formula $-O-P-(NR^8R^{8*})-R^9$, where R^8 and R^{8*} independently are selected among linear or branched optional substituted C_{1-6}-alkyl and C_{1-6}-alkenyl, and R^9 is a phosphate protection group. Each of the substituents R^{1*}, R^{2*}, R^{3*},R^4, R^5, R^{5*}, R^6, R^{6*}, R^7, and R^{7*} is independently selected from hydrogen, optionally substituted linear or branched C_{1-12}-alkyl, optionally substituted linear or branched C_{2-12}-alkenyl, optionally substituted linear or branched C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl,

aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-aminocarbonyl, amino-C_{1-6}-alkylaminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkylcarbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, azido, sulfanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradi-cal consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)-, where R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and the compound of formula II where the A and the OM group optionally have changed place. The compound of the formula II may also comprise basic salts and acid addition salts thereof. For brevity, the above intermediate may be referred to as "α-L-RNA amidite".

The general formula II may comprise a 5- or 6-membered ring as a part of the nucleoside structure. Five membered rings are desirable in some applications because they are able to occupy essentially the same conformations as the native furanose ring of a naturally occurring nucleoside. Among the possible 5-membered rings, the embodiments, wherein X is selected from the group consisting of –O-, -S-, -S(O)-, -$S(O)_2$-, and –$N(R^{N*})$- are desirable. In particular, the aforementioned embodiment of the invention pertaining to use of –O- is interesting because the furanose ring is then identical to the one found in nature.

The intermediate wherein R^{1*}, R^{2*}, R^{3*}, R^4, R^5, or R^{5*} independently represent hydrogen, C_{1-4}-alkyl or C_{1-4}-alkoxy, notably hydrogen, is particularly desirable.

The hydroxy protection group M may be selected among any group having the ability to selectively protect a hydroxy group, e.g., a protecting group that ensures non-reactivity, in basic and neutral medium and which simultaneously has the ability to be removed (e.g., the ability to be removed in a suitable acidic medium) in preference to other protecting groups. Desirably, the hydroxy protection group M is selected from trityl, 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT); 9-(9-phenyl)xanthenyl (pixyl); ethoxycarbonyloxy, phenylazo-phenyloxycarbonyloxy, tetrahydropyranyl (THP), 9-fluorenyl-methoxycarbonyl (Fmoc), methoxytetrahydropyranyl (MTHP); trimethylsilyl (TMS), triisopropylsilyl (TIPS), tert-butyldimethylsilyl (TBDMS), triethylsilyl, phenyldimethylsilyl; benzyloxycarbonyl; 2-bromobenzyloxycarbonyl; tert-butyl ethers; methyl ether; acetals; acetyl; or halogen substituted acetyls, e.g. chloroacetyl or fluoroacetyl; isobu-tyryl; pivaloyl; benzoyl and substituted benzoyls; methoxymethyl (MOM); and benzyl ethers or substituted benzyl ethers, including 2,6-dichlorobenzyl (2,6-Cl_2Bn).

The protection group L may be selected among groups having the ability to selectively protect, e.g., ensure non-reactivity of, a hydroxy protecting group in certain acid or neutral media, while allowing for removal in an appropriate medium, desirably in preference to other protecting groups. Desirable substituents L include 2'-O-triisopropylsilyloxymethyl (TOM) and 2'-O-t-butyldimethylsilyl (TBDMS). When the oligonucleotide synthesis is completed, TBDMS and TOM may be removed with tetrabutylammoniumfluoride (TBAF) or another suitable deprotecting reagent.

In some applications, it may be desired to synthesize the oligonucleotide in the reverse direction, that is from the 5' end to the 3' end. To this end, a so-

called reverse amidite is used. In a scheme using a reverse amidite, the A and the OM group have changed places, i.e., the A substituent is arranged at the 5' position of the formula of the general formula II, and the OM substituent is at the 3' position. For example, A may be hydroxy or protected hydroxy, and M may be -P-(NR^8R^{8*})-R^9.

The substituent B comprises a variety of possible moieties, which may contain chemical groups reactive under the conditions prevailing in chemical oligonucleotide synthesis. Such reaction sensitive groups are desirably protected to keep side reactions to a mininum.

In the event B is a protected nucleobase, the nucleobase is suitably selected among cytosin-1-yl having the amino group at the 4 position protected, adenin-9-yl having the amino group at the 6 position protected, and gaunin-9-yl having the amino group at the 2 position. The amino protecting group may be independently selected among acetyl (Ac), phenoxyacetyl (Pac), isopropylphenoxyacetyl (iPr-Pac), benzoyl (Bz), and dimethylformamidine (Dmf).

In a desired aspect, the optionally protected nucleobase is selected from the group consisting of 4-*N*-acetylcytosin-1-yl, 6-*N*-phenoxyacetyladenin-9-yl, 6-*N*-benzoyladenin-9-yl, 2-*N*-isopropylphenoxyacetylgaunin-9-yl, 2-*N*-dimethylform-amidinegaunin-9-yl, thymin-1-yl, 5-methyl-5-*N*-benzoyl-cytosine, 5-*N*-benzoylcytosine, and uracil-1-yl.

The phosphate protection group R^9 is selected among groups having the ability to remain in position under acid conditions, while being removed under basic conditions. Desirable phosphate protection groups are selected from 2-cyanoethyloxy, 2,2,2-trichloro-1,1-dimethyl ethyloxy, *p*-nitrophenylethyloxy, methoxy, methylthio, and allyloxy.

Methods for Synthesis of Nucleic Acids on a Solid Support

In another aspect, the invention provides a method for the synthesis of a population of nucleic acids (e.g., a population of nucleic acids of the invention) on a solid support. This method involves the reaction of a plurality of nucleoside phosphoramidites with an activated solid support (e.g., a solid support with an activated linker) and the subsequent reaction of a plurality of nucleoside phosphoramidites with activated nucleotides or nucleic acids bound to the solid support.

In some embodiments of the above aspect, the solid support or the growing nucleic acid bound to the solid support is activated by illumination, a photogenerated acid, or electric current. In desirable embodiments, one or more spots or regions (e.g., a region with an area of less than 1 cm^2, 0.1 cm^2, 0.01 cm^2, 1 mm^2, or 0.1 mm^2 that desirably contains one particular nucleic acid monomer or oligomer) on the solid support are irradiated to produce a photogenerated acid that removes the 5'-OH protecting group of one or more nucleic acid monomers or oligomers to which a nucleotide is subsequently added. In other embodiments, an electric current is applied to one or more spots or regions (e.g., a region with an area of less than 1 cm^2, 0.1 cm^2, 0.01 cm^2, 1 mm^2, or 0.1 mm^2 that desirably contains one particular nucleic acid monomer or oligomer) on the solid support to remove an electrochemically sensitive protecting group of one or more nucleic acid monomers or oligomers to which a nucleotide is subsequently added. In still other embodiments, one or more spots or regions (e.g., a region with an area of less than 1 cm^2, 0.1 cm^2, 0.01 cm^2, 1 mm^2, or 0.1 mm^2 that desirably contains one particular nucleic acid monomer or oligomer) on the solid support are irradiated to remove a photosensitive protecting group of one or more nucleic acid monomers or oligomers to which a nucleotide is subsequently added. In various embodiments, the solid support (e.g., chip, coverslip, microscope glass slide, quartz, or silicon) is less than 1, 0.5, 0.1, or 0.05 mm thick.

The present invention also pertains to a process for the production of an oligonucleotide comprising at least one α-L-RNA monomer, the process includes the steps of (a) providing a nucleoside unit attached to a solid support through a base-labile bond, the nucleoside being protected on the 5'-position with an acid-labile group; (b) treating the solid support, attached to a nucleoside according to step (a) or (g), with an acid to remove the 5'-protection group; (c) adding a proton donating activator and a successive nucleoside monomer comprising at the 3'-position a phosphoramidite group and at the 5'-position an acid-labile protection group; (d) reacting the nucleoside attached to the solid support with the successive nucleoside phosphoramidite derivative to produce a phosphite-triester linkage between the 5' position of the nucleoside attached to the solid support and the 3' position of the successive nucleoside monomer; (e) optionally, capping any unreacted 5'-hydroxy groups; (f) adding an oxidizing agent to convert the phosphite triester group to a phosphodiester group; (g) subjecting the oxidized product of step (f) to the steps (b) through (f) or; (h) adding a base to detach the support and any protection groups, thus releasing the oligonucleotide). At least one of the nucleoside monomers of step (c) are selected from the α-L-RNA intermediates disclosed above.

In the process of preparing an oligonucleotide with at least one α-L-RNA monomer, one, more, or all hydroxy and exocyclic amino group(s) of the first nucleoside attached to the support, and any subsequently added nucleoside monomer, may be protected. Desirably, all the groups prone to reaction under the conditions prevailing during the synthesis of the oligo-nucleotide are protected. To remove the 5' protection group, the nucleoside directly attached to the solid support or the nucleotide indirectly attached to the solid support through one or more nucleotide monomer(s), an acid is added. Desirably, the acid is 2,2-dichloroacetic acid or 2,2,2-trichloroacetic acid. The reaction medium of step (b) is, in general, a non-aquaeous media, such as dichloromethane, trichloromethane, or toluene. In addition, the proton

donating activator of step (c) is desirably 1*H*-tetrazole, 4,5-dicyano imidazole or a pyridinium salt. To ensure that oligonucleotides comprising un-reacted 5'-hydroxy groups are not participating in the subsequent reaction cycle, any free hydroxy group is optionally capped. In a desirable embodiment, the capping of 5'-hydroxy groups in step (e) is accomplished by reacting the 5'-hydroxy groups with acetic anhydride for the formation of acetyl groups. Desirably, *N*-methylimidazole is also present during the reaction. The sequence of a cycle is usually terminated with an oxidation step to oxidize the phosphite group to the corresponding phosphate group. A suitable oxidizing agent is iodine.

Pharmaceutical Compositions and Nucleic Acid Populations

In another aspect, the invention features a pharmaceutical composition that includes one or more of the nucleic acids and/or alpha-L-RNA monomers of the invention (e.g., an α-L-RNA nucleoside or nucleotide) and a pharmaceutically acceptable carrier, such as one of the carriers described herein.

In another aspect, the invention features a population of two or more nucleic acids of the invention. The populations of nucleic acids of the invention may contain any number of unique molecules. For example, the population may contain as few as 10, 10^2, 10^4, or 10^5 unique molecules or as many as 10^7, 10^8, 10^9 or more unique molecules. In desirable embodiments, at least 1, 5, 10, 50, 100 or more of the polynucleotide sequences are a non-naturally-occurring sequence. Desirably, at least 20, 40, or 60% of the unique polynucleotide sequences are non-naturally-occurring sequences. Desirably, the nucleic acids are all the same length; however, some of the molecules may differ in length.

Desirable Nucleic Acids for Any of the Above Aspects

In desirable embodiments of any of the above aspects, the length of one or more nucleic acids (e.g., nucleic acids in a nucleic acid population of the invention) is between 15 and 150 nucleotides, 5 and 100 nucleotides, 20 and 80 nucleotides, or 30 and 60 nucleotides in length, inclusive. In particular embodiments, the nucleic acid is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 nucleotides or at least 60, 70, 80, 90, 100, 120, or 130 nucleotides in length.

In some embodiments, the oligonucleotide has one or more nucleotides with universal bases. For example, nucleotides with universal bases can be used to increase the thermal stability of the oligonucleotides. In some embodiments, the oligonucleotide has a universal base located at the 5' or 3' terminus of the nucleic acid. In desirable embodiments, one or more (e.g., 2, 3, 4, 5, 6, or more) universal bases are located at the 5' and 3' termini of the oligonucleotide. Desirably, all of the oligonucleotides in the population have the same number of universal bases. Desirable universal bases include inosine, 3-nitropyrrole, 5-nitroindole, pyrene and pyridyloxazole derivatives, pyrenyl, pyrenylmethylglycerol moieties, pyrrole, diazole or triazole moieties, all of which may be optionally substituted, and other groups e.g. modified adenine, cytosine, 5-methylcytosine, isocytosine, pseudoisocytosine, guanine, thymine, uracil, 5-bromouracil, 5-propynyluracil, 5-propyny-6-fluoroluracil, 5-methylthiazoleuracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 7-propyne-7-deazaadenine, 7-propyne-7-deazaguanine. Other desirable universal bases include, pyrrole, diazole or triazole moieties, all of which may be optionally substituted.

In some embodiments, the oligonucleotide has one or more nucleotides with universal bases. For example, nucleotides with universal bases can be

used to increase the thermal stability of the oligonucleotides. In some embodiments, the oligonucleotide has a universal base located at the 5' or 3' terminus of the nucleic acid. In desirable embodiments, one or more (e.g., 2, 3, 4, 5, 6, or more) universal bases are located at the 5' and 3' termini of the oligonucleotide. Desirably, all of the oligonucleotides in the population have the same number of universal bases. Desirable universal bases include inosine, 3-nitropyrrole, 5-nitroindole, pyrene and pyridyloxazole derivatives, pyrenyl, pyrenylmethylglycerol moieties, pyrrole, diazole or triazole moieties, all of which may be optionally substituted, and other groups e.g. modified adenine, cytosine, 5-methylcytosine, isocytosine, pseudoisocytosine, guanine, thymine, uracil, 5-bromouracil, 5-propynyluracil, 5-propyny-6-fluoroluracil, 5-methylthiazoleuracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 7-propyne-7-deazaadenine, 7-propyne-7-deazaguanine. Other desirable universal bases include, pyrrole, diazole or triazole moieties, all of which may be optionally substituted.

In still other embodiments, the nucleic acids are covalently bonded to a solid support. Desirably, the nucleic acids are in a predefined arrangement. In various embodiments, the first population has at least 10; 100; 1,000; 5,000; 10,000; 100,000; or 1,000,000 different nucleic acids.

Desirable LNA units have a carbon or hetero alicyclic ring with four to six ring members, e.g. a furanose ring, or other alicyclic ring structures such as a cyclopentyl, cycloheptyl, tetrahydropyranyl, oxepanyl, tetrahydrothiophenyl, pyrrolidinyl, thianyl, thiepanyl, piperidinyl, and the like. In one aspect, at least one ring atom of the carbon or hetero alicyclic group is taken to form a further cyclic linkage to thereby provide a multi-cyclic group. The cyclic linkage may include one or more, typically two atoms, of the carbon or hetero alicyclic group. The cyclic linkage also may include one or more atoms that are substituents, but not ring members, of the carbon or hetero alicyclic group.

Other desirable LNA units are compounds having a substituent on the 2'-position of the central sugar moiety (e.g., ribose or xylose), or derivatives thereof, which favors the C3'-endo conformation, commonly referred to as the North (or simply N for short) conformation. These LNA units include ENA (2'-O,4'-C-ethylene-bridged nucleic acids such as those disclosed in WO 00/47599) units as well as non-bridged riboses such as 2'-F or 2'-O-methyl.

Methods for Detecting or Amplifying Target Nucleic Acids

In one aspect, the invention features a method for detecting the presence of one or more target nucleic acids in a sample. This method involves incubating a nucleic acid sample with one or more nucleic acids of the invention under conditions that allow at least one target nucleic acid to hybridize to at least one of the nucleic acids of the invention. Desirably, hybridization is detected for at least 2, 3, 4, 5, 6, 8, 10, or 12 target nucleic acids. In some embodiments, the method further includes contacting the target nucleic acid with a second nucleic acid or a population of second nucleic acids that binds to a different region of the target molecule than the first nucleic acid. Desirably, the method further involves identifying one or more hybridized target nucleic acids and/or determining the amount of one or more hybridized target nucleic acids. In desirable embodiments, the method further includes determining the presence or absence of nucleic acid of interest (e.g., an mRNA) in the sample and/or determining the presence or absence of a mutation, deletion, and/or duplication in the nucleic acid of interest. In some embodiments, the mutation, deletion, and/or duplication is indicative of a disease, disorder, or condition, such as cancer.

In one aspect, the invention features a method of detecting a nucleic acid of a pathogen (e.g., a nucleic acid in a sample such as a blood or urine sample from a mammal). This method involves contacting a nucleic acid probe of the

invention (e.g., a probe specific for an mRNA from a particular pathogen or family of pathogens) with a nucleic acid sample under conditions that allow the probe to hybridize to at least one nucleic acid in the sample. The probe is desirably at least 60, 70, 80, 90, 95, or 100% complementary to a nucleic acid of a pathogen (e.g., a bacteria, virus, or yeast such as any of the pathogens described herein). Hybridization between the probe and a nucleic acid in the sample is detected, indicating that the sample contains the corresponding nucleic acid from a pathogen. In some embodiments, the method is used to determine what strain of a pathogen has infected a mammal (e.g., a human) by determining whether a particular nucleic acid is present in the sample. In other embodiments, the probe has a universal base in a position corresponding to a nucleotide that varies among different strains of a pathogen, and thus the probe detects the presence of a nucleic acid from any of a multiple of pathogenic strains.

Desirably, the method also includes identifying the hybridized target nucleic acid and/or determining the amount of hybridized target nucleic acid. In particular embodiments, the target nucleic acids are labeled with a fluorescent group. Desirably, the first nucleic acid standard is labeled with a different fluorescent group. The fluorescence of the target nucleic acids and the first nucleic acid standard can be detected simultaneously or sequentially.

In another aspect, the invention features a method for amplifying a target nucleic acid molecule. The method involves (a) incubating a first nucleic acid of the invention with a target nucleic acid under conditions that allow the first nucleic acid to bind the target nucleic acid; and (b) extending the first nucleic acid with the target nucleic acid as a template. Desirably, the method further involves contacting the target nucleic acid with a second nucleic acid (e.g., a second nucleic acid of

the invention) that binds to a different region of the target nucleic acid than the first nucleic acid. In various embodiments, the sequence of the target nucleic acid is known or unknown.

Methods for Silencing a Target Nucleic Acid in a Cell or Animal

One method for inhibiting specific gene expression involves the use of antisense or double stranded oligonucleotides which are complementary to a specific target nucleic acid (e.g., an mRNA). Of special interest are oligonucleotides with a modified backbone (such as LNA or phosphorothioate) that are not readily degraded by endonucleases in the target cells.

In one aspect, the invention provides a method for inhibiting the expression of a target nucleic acid in a cell. The method involves introducing into the cell a nucleic acid of the invention in an amount sufficient to specifically attenuate expression of the target nucleic acid. The introduced nucleic acid has a nucleotide sequence that is essentially complementary to a region of desirably at least 20 nucleotides of the target nucleic acid. Desirably, the cell is in a human.

In a related aspect, the invention provides a method for preventing, stabilizing, or treating a disease, disorder, or condition associated with a target nucleic acid in a mammal (e.g., a human patient). This method involves introducing into the mammal (e.g., a human patient) a nucleic acid of the invention in an amount sufficient to specifically attenuate expression of the target nucleic acid, wherein the introduced nucleic acid has a nucleotide sequence that is essentially complementary to a region of desirably at least 20 nucleotides of the target nucleic acid.

In another aspect, the invention provides a method for preventing, stabilizing, or treating a pathogenic infection in a human patient by introducing into said patient a nucleic acid of the invention in an amount sufficient to specifically attenuate expression of a target nucleic acid of a pathogen. The

introduced nucleic acid has a nucleotide sequence that is essentially complementary to a region of desirably at least 20 nucleotides of the target nucleic acid.

The oligonucleotide according to the present invention may be used for a variety of applications. The ability of the nucleotide to discriminate between DNA and RNA enhances its use for antisense or other gene silencing technology. The present invention therefore includes the use of an oligonucleotide according to the invention for the manufacture of a pharmaceutical composition for the treatment, stabilization, or prevention of a disease, disorder, or infection. Similarly, the invention includes a method of treating a subject having, or suspected of having, a disease, disorder, or infection comprising administering to the subject an amount of an oligonucleotide of the invention that which is effective to treat, stabilize, or prevent the disease, disorder, or infection.

Most desirable is the treatment of human disease by the oligonucleotide of the present invention. Exemplary conditions that can be treated or prevented in humans include acute hepatic failure, autoimmune disorders, blood disorders, bone disorders, cancer, including bladder cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, cancer of the head and neck, lung cancer, metastatic cancer, liver cancer, leukemia, ovarian cancer, prostate cancer, renal cell carcinoma, sarcoma, and skin cancer, cardiovascular disease, gastrointestinal tract disorders, infectious disease, including HIV, inherited autosomal disease, mesothelioma, myopathies, neurological disorders, and neuropathy. In some embodiments, the introduced nucleic acid is single stranded or double stranded.

With respect to the therapeutic methods of the invention, it is not intended that the administration of nucleic acids to a mammal (e.g., a patient) be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration,

including oral, intraperitoneal, intramuscular, intravenous, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to prevent or treat a disease (e.g., a disease associated with the expression of a target nucleic acid that is silenced with a nucleic acid of the invention). One or more nucleic acids may be administered in a single dose or multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one week, one month, one year, or ten years. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

Optimum dosages for gene silencing applications may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50} values found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.001 ug to 100 g per kg of body weight (e.g., 0.001 ug/kg to 1 g/kg), and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years (U.S.P.N. 6,440,739). Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.001 ug to 100 g per kg of body weight (e.g., 0.001 ug/kg to 1 g/kg), once or more daily, to once every 20 years. If desired, conventional treatments may be used in combination with the nucleic acids of the present invention.

Suitable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The composition can be adapted for the mode of administration and can be in the form of, for

example, a pill, tablet, capsule, spray, powder, or liquid. In some embodiments, the pharmaceutical composition contains one or more pharmaceutically acceptable additives suitable for the selected route and mode of administration. These compositions may be administered by, without limitation, any parenteral route including intravenous, intra-arterial, intramuscular, subcutaneous, intradermal, intraperitoneal, intrathecal, as well as topically, orally, and by mucosal routes of delivery such as intranasal, inhalation, rectal, vaginal, buccal, and sublingual. In some embodiments, the pharmaceutical compositions of the invention are prepared for administration to vertebrate (e.g., mammalian) subjects in the form of liquids, including sterile, non-pyrogenic liquids for injection, emulsions, powders, aerosols, tablets, capsules, enteric coated tablets, or suppositories.

In another aspect, the invention features a method of modulating the ability of a target oligonucleotide to act as a substrate for one or more nucleic acid enzymes. This method involves hybridizing an oligonucleotide of invention to a target oligonucleotide. In a related aspect, one or more α-L-RNA monomers with the oligonucleotide modulate the ability of the oligonucleotide to act as a substrate for nucleic acid active enzymes. Desirably, the oligonucleotide has a region with substantial complementarity to a target oligonucleotide and also has a region of nucleotides that recruits a nucleic acid active enzyme, such as RNaseRNase H. Particularly desirable is when the oligonucleotide of the invention consists of DNA monomers and at least one α-L-RNA monomer and the target oligonucleotide is a single-stranded or double-stranded RNA sequence, the hybridization of which by the oligonucleotide of the invention results in a sequence-specific strand displacement. Most desirable is when the oligonucleotide of the invention is 5 -d(GTC TCT A($^{\alpha L}$U)G GAC CT), 5 -d(GTC ($^{\alpha L}$U)CT ATG GAC CT), or 5 -d(G($^{\alpha L}$U)C TCT ATG GAC CT). The combination of the selective hybridization of the oligonucleotide of the invention to a target gene to be silenced and the

decomposition of the complementary strand by RNase H can be a powerful tool for antisense applications.

The selective hybridization of the oligonucleotides of the invention to RNA also can find usage in the purification of RNA (e.g., mRNA) from a sample. In one such method for purifying RNA from a sample, an oligonucleotide that has a region with substantial complementarity to a corresponding region in an RNA of interest is contacted with the RNA of interest under conditions that allow hybridization between the oligonucleotide and the RNA of interest. The hybridized RNA is isolated. Desirably, the isolation step includes a method selected from the group consisting of filtration, affinity chromatography, ion exchange chromatography, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, chromatofocusing, centrifugation, high pressure liquid chromatography, and dialysis. In one embodiment, the oligonucleotide of the invention is bound to a solid support. Especially contemplated is a one-step recovery of the mRNA from a sample, e.g., as disclosed in US patent No. 6,303,315 (One step sample preparation and detection of nucleic acids in complex biological samples) which is incorporated herein by reference.

In another aspect, the invention features a method for binding an oligonucleotide to a target squence in a dsRNA molecule. An oligonucleotide of the invention is contacted with a dsRNA molecule under conditions that allow the oligonucleotide to hybridize or bind to the dsRNA molecule by way of strand displacement or triple helix formation.

In another aspect of the invention, the resistance of an analyzing or purification system to degradation by one or more nucleic acid-active enzymes is increased by administering an oligonucleotide of the invention to the analyzing or purification system.

In another aspect of the invention, the selective hybridization of the oligonucleotides of the invention to RNA can also be used to detect one or

more mismatches in a target RNA sequence when the oligonucleotide comprises a region with substantial complementarity to the corresponding region in the target RNA and, when hybridized to the target RNA oligonucleotide having one or more mismatches, exhibits a reduction in T_m compared to the T_m obtained for the hybridization of the oligonucleotide of the invention to an RNA oligonucleotide that has 100% complementarity.

Oligonucleotides have been described not only as catalysts in self-modifiifying biological reactions, but also in such diverse reactions a Diels-Alder reactions, glycosidic bond formations, alkylations, acylations, amide bond formations, or in the hydrolysis of phosphodiester linkages or bonds. Therefore, as an additional aspect, the oligonucleotides of the invention can be used as a catalyst in a biological or chemical process (e.g., a catalyst for the hydrolysis of a phosphodiester linkage or bond). In one such aspect, the invention provides a method for catalyzing a biological or chemical reaction. This method involves administering an oligonucleotide of the invention to a reaction mixture in an amount sufficient to increase the rate of the reaction.

Desirable Embodiments of Any of the Aspects of the Invention

In other embodiments of any of various aspects of the invention, a nucleic acid probe or primer specifically hybridizes to a target nucleic acid but does not substantially hybridize to non-target molecules, which include other nucleic acids in a cell or biological sample having a sequence that is less than 99, 95, 90, 80, or 70% identical or complementary to that of the target nucleic acid. Desirably, the amount of the these non-target molecules hybridized to, or associated with, the nucleic acid probe or primer, as measured using standard assays, is 2-fold, desirably 5-fold, more desirably 10-fold, and most desirably 50-fold lower than the amount of the target nucleic acid hybridized to, or associated with, the nucleic acid probe or primer. In other embodiments, the amount of a target nucleic acid hybridized to, or associated with, the nucleic

acid probe or primer, as measured using standard assays, is 2-fold, desirably 5-fold, more desirably 10-fold, and most desirably 50-fold greater than the amount of a control nucleic acid hybridized to, or associated with, the nucleic acid probe or primer. In certain embodiments, the nucleic acid probe or primer RNA is substantially complementary (*e.g.*, at least 80, 90, 95, 98, or 100% complementary) to a target nucleic acid or a group of target nucleic acids from a cell. In other embodiments, the probe or primer is homologous to multiple RNA or DNA molecules, such as RNA or DNA molecules from the same gene family. In other embodiments, the probe or primer is homologous to a large number of RNA or DNA molecules. In desirable embodiments, the probe or primer binds to nucleic acids which have polynucleotide sequences that differ in sequence at a position that corresponds to the position of a universal base in the probe or primer. Examples of control nucleic acids include nucleic acids with a random sequence or nucleic acids known to have little, if any, affinity for the nucleic acid probe or primer. In some embodiments, the target nucleic acid is an RNA, DNA, or cDNA molecule.

Definitions

When used herein, the term "LNA" (Locked Nucleoside Analogues) refers to nucleoside analogues (e.g., bicyclic nucleoside analogues, e.g., as disclosed in WO 9914226) either incorporated in an oligonucleotide or as a discrete chemical species (e.g., LNA nucleoside and LNA nucleotide). The term "monomeric LNA" may, e.g., refer to the monomers LNA A, LNA T, LNA C, or any other LNA monomers.

By "LNA unit" is meant an individual LNA monomer (e.g., an LNA nucleoside or LNA nucleotide) or an oligomer (e.g., an oligonucleotide or nucleic acid) that includes at least one LNA monomer. LNA units as disclosed in WO 99/14226,WO 0056746, WO 0056748, and WO 0066604 are in general particularly desirable modified nucleic acids for incorporation into an

oligonucleotide of the invention and includes ENA (2'O,4'C-ethylene-bridged nucleic acids). Additionally, the nucleic acids may be modified at either the 3' and/or 5' end by any type of modification known in the art. For example, either or both ends may be capped with a protecting group, attached to a flexible linking group, attached to a reactive group to aid in attachment to the substrate surface, etc. Desirable LNA units and their method of synthesis also are disclosed in WO 0056746, WO 0056748, WO 0066604, Morita *et al.*, Bioorg. Med. Chem. Lett. 12(1):73-76, 2002; Hakansson *et al.*, Bioorg. Med. Chem. Lett. 11(7):935-938, 2001; Koshkin *et al.*, J. Org. Chem. 66(25):8504-8512, 2001; Kvaerno *et al.*, J. Org. Chem. 66(16):5498-5503, 2001; Hakansson *et al.*, J. Org. Chem. 65(17):5161-5166, 2000; Kvaerno *et al.*, J. Org. Chem. 65(17):5167-5176, 2000; Pfundheller *et al.*, Nucleosides Nucleotides 18(9):2017-2030, 1999; and Kumar *et al.*, Bioorg. Med. Chem. Lett. 8(16):2219-2222, 1998.

Exemplary 5', 3', and/or 2' terminal groups include -H, -OH, halo (*e.g.*, chloro, fluoro, iodo, or bromo), optionally substituted aryl, (*e.g.*, phenyl or benzyl), alkyl (*e.g*, methyl or ethyl), alkoxy (*e.g.*, methoxy), acyl (*e.g.* acetyl or benzoyl), aroyl, aralkyl, hydroxy, hydroxyalkyl, alkoxy, aryloxy, aralkoxy, nitro, cyano, carboxy, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, acylamino, aroylamine, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, alkylsulfinyl, arylsulfinyl, heteroarylsulfinyl, alkylthio, arylthio, heteroarylthio, aralkylthio, heteroaralkylthio,amidino, amino, carbamoyl, sulfamoyl, alkene, alkyne, protecting groups (e.g., silyl, 4,4'-dimethoxytrityl, monomethoxytrityl, or trityl(triphenylmethyl)), linkers (e.g., a linker containing an amine, ethylene glycol, quinone such as anthraquinone), detectable labels (e.g., radiolabels or fluorescent labels), and biotin.

A "modified base" or other similar term refers to a composition (e.g., a non-naturally occuring nucleobase or nucleosidic base) which can pair with a natural base (e.g., adenine, guanine, cytosine, uracil, and/or thymine) and/or

can pair with a non-naturally occurring nucleobase or nucleosidic base. Desirably, the modified base provides a T_m differential of 15, 12, 10, 8, 6, 4, or 2°C or less as described herein. Exemplary modified bases are described in EP 1 072 679 and WO 97/12896.

By "nucleobase" is meant the naturally occurring nucleobases adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) as well as non-naturally occurring nucleobases such as xanthine, diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine (mC), 5-(C^3-C^6)-alkynyl-cytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanine, inosine and the "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol. 25, pp 4429-4443. The term "nucleobase" thus includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and tautomers thereof. Further naturally and non-naturally occurring nucleobases include those disclosed in U.S. Pat. No. 3,687,808 (Merigan, et al.), in Chapter 15 by Sanghvi, in Antisense Research and Application, Ed. S. T. Crooke and B. Lebleu, CRC Press, 1993, in Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613-722 (see especially pages 622 and 623, and in the Concise Encyclopedia of Polymer Science and Engineering, J. I. Kroschwitz Ed., John Wiley & Sons, 1990, pages 858-859, Cook, Anti-Cancer Drug Design 1991, 6, 585-607, each of which are hereby incorporated by reference in their entirety). The term "nucleosidic base" or "base unit" is further intended to include compounds such as heterocyclic compounds that can serve like nucleobases including certain "universal bases" that are not nucleosidic b.ases in the most classical sense but serve as nucleosidic bases. Especially mentioned as universal bases are 3-nitropyrrole, optionally substituted indoles

(e.g., 5-nitroindole), and optionally substituted hypoxanthine. Other desirable universal bases include, pyrrole, diazole or triazole derivatives, including those universal bases known in the art.

As described herein, various groups of an LNA unit may be optionally substituted. A "substituted" group such as a nucleobase or nucleosidic base and the like may be substituted by other than hydrogen at one or more available positions, typically 1 to 3 or 4 positions, by one or more suitable groups such as those disclosed herein. Suitable groups that may be present on a "substituted" group include e.g. halogen such as fluoro, chloro, bromo and iodo; cyano; hydroxyl; nitro; azido; alkanoyl such as a C_{1-6} alkanoyl group such as acyl and the like; carboxamido; alkyl groups including those groups having 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to 12 carbon, or 2, 3, 4, 5 or 6 carbon atoms; alkoxy groups including those having one or more oxygen linkages and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those moieties having one or more thioether linkages and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; alkylsulfinyl groups including those moieties having one or more sulfinyl linkages and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; alkylsulfonyl groups including those moieties having one or more sulfonyl linkages and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; aminoalkyl groups such as groups having one or more N atoms and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; carbocyclic aryl having 6 or more carbons; aralkyl having 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, with benzyl being a desirable group; aralkoxy having 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, with O-benzyl being a desirable group; or a heteroaromatic or heteroalicyclic group having 1 to 3 separate or fused rings with 3 to about 8 members per ring and one or more N, O or S

atoms, e.g. coumarinyl, quinolinyl, pyridyl, pyrazinyl, pyrimidyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, indolyl, benzofuranyl, benzothiazolyl, tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino and pyrrolidinyl.

By "universal base" is meant a naturally-occurring or desirably a non-naturally occurring compound or moiety that can pair with a natural base (e.g., adenine, guanine, cytosine, uracil, and/or thymine), and that has a T_m differential of 15, 12, 10, 8, 6, 4, or 2°C or less as described herein.

The term " T_m " is used in reference to the "melting temperature." The melting temperature is the temperature at which 50% of a population of double-stranded nucleic acid molecules becomes dissociated into single strands. The equation for calculating the T_m of nucleic acids is well-known in the art. The T_m of a hybrid nucleic acid is often estimated using a formula adopted from hybridization assays in 1 M salt, and commonly used for calculating T_m for PCR primers: T_m =[(number of A+T) x 2°C + (number of G+C) x 4 °C]. C. R. Newton et al. PCR, 2nd Ed., Springer-Verlag (New York: 1997), p. 24. This formula was found to be inaccurate for primers longer that 20 nucleotides. Id. Other more sophisticated computations exist in the art which take structural as well as sequence characteristics into account for the calculation of T_m. A calculated T_m is merely an estimate; the optimum temperature is commonly determined empirically.

A nucleic acid compound that has a T_m differential of a specified amount (e.g., less than 15, 12, 10, 8, 6, 4, 2, or 1°C) means the nucleic acid exhibits that specified T_m differential when incorporated into a specified 9-mer oligonucleotide with respect to the four complementary variants, as defined immediately below.

Unless otherwise indicated, a T_m differential provided by a particular modified base is calculated by the following protocol (steps a) through d)):

a) incorporating the modified base of interest into the following oligonucleotide 5'-d(GTGAMATGC), wherein **M** is the modified base;

b) mixing 1.5 x 10^{-6}M of the oligonucleotide having incorporated therein the modified base with each of 1.5x10^{-6}M of the four oligonucleotides having the sequence 3'-d(CACTYTACG), wherein **Y** is A, C, G, T, respectively, in a buffer of 10mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0;

c) allowing the oligonucleotides to hybridize; and

d) detecting the T_m for each of the four hybridized nucleotides by heating the hybridized nucleotides and observing the temperature at which the maximum of the first derivative of the melting curve recorded at a wavelength of 260 nm is obtained.

Unless otherwise indicated, a T_m differential for a particular modified base is determined by subtracting the highest T_m value determined in steps a) through d) immediately above from the lowest T_m value determined by steps a) through d) immediately above.

Monomers are referred to as being "complementary" if they contain nucleobases that can form hydrogen bonds according to Watson-Crick base-pairing rules (e.g., G with C, A with T, or A with U) or other hydrogen bonding motifs such as for example diaminopurine with T, inosine with C, and pseudoisocytosine with G.

By "substantially complementarity" is meant having a sequence that is at least 60, 70, 80, 90, 95, or 100% complementary to that of another sequence. Sequence complementarity is typically measured using sequence analysis software with the default parameters specified therein (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

The term "homology" refers to a degree of complementarity. There can be partial homology or complete homology (i.e., identity). A partially complementary sequence that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid is referred to using the functional term "substantially homologous."

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to a probe that can hybridize to a strand of the double-stranded nucleic acid sequence under conditions of low stringency, e.g. using a hybridization buffer comprising 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to a probe that can hybridize to (i.e., is the complement of) the single-stranded nucleic acid template sequence under conditions of low stringency, e.g. using a hybridization buffer comprising 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C.

By "corresponding unmodified reference nucleobase" is meant a nucleobase that is not part of an LNA unit and is in the same orientation as the nucleobase in an LNA unit.

By "mutation" is meant an alteration in a naturally-occurring or reference nucleic acid sequence, such as an insertion, deletion, frameshift mutation, silent mutation, nonsense mutation, or missense mutation. Desirably, the amino acid sequence encoded by the nucleic acid sequence has at least one amino acid alteration from a naturally-occurring sequence.

By "selecting" is meant substantially partitioning a molecule from other molecules in a population. Desirably, the partitioning provides at least a 2-fold,

desirably, a 30-fold, more desirably, a 100-fold, and most desirably, a 1,000-fold enrichment of a desired molecule relative to undesired molecules in a population following the selection step. The selection step may be repeated a number of times, and different types of selection steps may be combined in a given approach. The population desirably contains at least 10^9 molecules, more desirably at least 10^{11}, 10^{13}, or 10^{14} molecules and, most desirably, at least 10^{15} molecules.

By a "population" is meant more than one nucleic acid. A "population" according to the invention desirably means more than 10^1, 10^2, 10^3, or 10^4 different molecules.

By "target nucleic acid" or "nucleic acid target" is meant a particular nucleic acid sequence of interest. Thus, the "target" can exist in the presence of other nucleic acid molecules or within a larger nucleic acid molecule.

By "solid support" is meant any rigid or semi-rigid material to which a nucleic acid binds or is directly or indirectly attached. The support can be any porous or non-porous water insoluble material, including without limitation, membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, strips, plates, rods, polymers, particles, microparticles, capillaries, and the like. The support can have a variety of surface forms, such as wells, trenches, pins, channels and pores.

By an "array" is meant a fixed pattern of at least two different immobilized nucleic acids on a solid support. Desirably, the array includes at least 10^2, more desirably, at least 10^3, and, most desirably, at least 10^4 different nucleic acids.

By "antisense nucleic acid" is meant a nucleic acid, regardless of length, that is complementary to a coding strand or mRNA of interest. In some embodiments, the antisense molecule inhibits the expression of only one nucleic acid, and in other embodiments, the antisense molecule inhibits the expression of more than one nucleic acid. Desirably, the antisense nucleic acid

decreases the expression or biological activity of a nucleic and or encoded protein by at least 20, 40, 50, 60, 70, 80, 90, 95, or 100%. An antisense molecule can be introduced, e.g., to an individual cell or to whole animals, for example, it may be introduced systemically via the bloodstream. Desirably, a region of the antisense nucleic acid or the entire antisense nucleic acid is at least 70, 80, 90, 95, 98, or 100% complimentary to a coding sequence, regulatory region (5' or 3' untranslated region), or an mRNA of interest. Desirably, the region of complementarity includes at least 5, 10, 20, 30, 50, 75,100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in the antisense nucleic acid.

In some embodiments, the antisense molecule is less than 200, 150, 100, 75, 50, or 25 nucleotides in length. In other embodiments, the antisense molecule is less than 50,000; 10,000; 5,000; or 2,000 nucleotides in length. In certain embodiments, the antisense molecule is at least 200, 300, 500, 1000, or 5000 nucleotides in length. In some embodiments, the number of nucleotides in the antisense molecule is contained in one of the following ranges: 5-15 nucleotides, 16-20 nucleotides, 21-25 nucleotides, 26-35 nucleotides, 36-45 nucleotides, 46-60 nucleotides, 61-80 nucleotides, 81-100 nucleotides, 101-150 nucleotides, or 151-200 nucleotides, inclusive. In addition, the antisense molecule may contain a sequence that is less than a full-length sequence or may contain a full-length sequence.

By "double stranded nucleic acid" is meant a nucleic acid containing a region of two or more nucleotides that are in a double stranded conformation. In various embodiments, the double stranded nucleic acids consists entirely of LNA units or a mixture of LNA units, ribonucleotides, and/or deoxynucleotides. The double stranded nucleic acid may be a single molecule with a region of self-complimentarity such that nucleotides in one segment of the molecule base-pair with nucleotides in another segment of the molecule. Alternatively, the double stranded nucleic acid may include two different

strands that have a region of complimentarity to each other. Desirably, the regions of complimentarity are at least 70, 80, 90, 95, 98, or 100% complimentary. Desirably, the region of the double stranded nucleic acid that is present in a double stranded conformation includes at least 5, 10, 20, 30, 50, 75,100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in the double stranded nucleic acid. Desirable double stranded nucleic acid molecules have a strand or region that is at least 70, 80, 90, 95, 98, or 100% identical to a coding region or a regulatory sequence (*e.g.*, a transcription factor binding site, a promoter, or a 5' or 3' untranslated region) of a nucleic acid of interest. In some embodiments, the double stranded nucleic acid is less than 200, 150, 100, 75, 50, or 25 nucleotides in length. In other embodiments, the double stranded nucleic acid is less than 50,000; 10,000; 5,000; or 2,000 nucleotides in length. In certain embodiments, the double stranded nucleic acid is at least 200, 300, 500, 1000, or 5000 nucleotides in length. In some embodiments, the number of nucleotides in the double stranded nucleic acid is contained in one of the following ranges: 5-15 nucleotides, 16-20 nucleotides, 21-25 nucleotides, 26-35 nucleotides, 36-45 nucleotides, 46-60 nucleotides, 61-80 nucleotides, 81-100 nucleotides, 101-150 nucleotides, or 151-200 nucleotides, inclusive. In addition, the double stranded nucleic acid may contain a sequence that is less than a full-length sequence or may contain a full-length sequence.

In some embodiments, the double stranded nucleic acid inhibits the expression of only one nucleic acid, and in other embodiments, the double stranded nucleic acid molecule inhibits the expression of more than one nucleic acid. Desirably, the nucleic acid decreases the expression or biological activity of a nucleic acid of interest or a protein encoded by a nucleic acid of interest by at least 20, 40, 50, 60, 70, 80, 90, 95, or 100%. A double stranded nucleic acid can be introduced, e.g., to an individual cell or to whole animals, for example, it may be introduced systemically via the bloodstream.

In various embodiments, the double stranded nucleic acid or antisense molecule includes one or more LNA nucleotides, one or more universal bases, and/or one or more modified nucleotides in which the 2' position in the sugar (e.g., riobse or xylose) contains a halogen (such as flourine group) or contains an alkoxy group (such as a methoxy group) which increases the half-life of the double stranded nucleic acid or antisense molecule *in vitro* or *in vivo* compared to the corresponding double stranded nucleic acid or antisense molecule in which the corresponding 2' position contains a hydrogen or an hydroxyl group. In yet other embodiments, the double stranded nucleic acid or antisense molecule includes one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. Desirably, the double strandwd or antisense molecule is purified.

By "purified" is meant separated from other components that naturally accompany it. Typically, a factor is substantially pure when it is at least 50%, by weight, free from proteins, antibodies, and naturally-occurring organic molecules with which it is naturally associated. Desirably, the factor is at least 75%, more desirably, at least 90%, and most desirably, at least 99%, by weight, pure. A substantially pure factor may be obtained by chemical synthesis, separation of the factor from natural sources, or production of the factor in a recombinant host cell that does not naturally produce the factor. Nucleic acids and proteins may be purified by one skilled in the art using standard techniques such as those described by Ausubel *et al.* (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 2000). The factor is desirably at least 2, 5, or 10 times as pure as the starting material, as measured using polyacrylamide gel electrophoresis, column chromatography, optical density, HPLC analysis, or western analysis (Ausubel *et al.*, *supra*). Desirable methods

of purification include immunoprecipitation, column chromatography such as immunoaffinity chromatography, magnetic bead immunoaffinity purification, and panning with a plate-bound antibody.

By "treating, stabilizing, or preventing a disease, disorder, or condition" is meant preventing or delaying an initial or subsequent occurrence of a disease, disorder, or condition; increasing the disease-free survival time between the disappearance of a condition and its reoccurrence; stabilizing or reducing an adverse symptom associated with a condition; or inhibiting or stabilizing the progression of a condition. Desirably, at least 20, 40, 60, 80, 90, or 95% of the treated subjects have a complete remission in which all evidence of the disease disappears. In another desirable embodiment, the length of time a patient survives after being diagnosed with a condition and treated with a nucleic acid of the invention is at least 20, 40, 60, 80, 100, 200, or even 500% greater than (i) the average amount of time an untreated patient survives or (ii) the average amount of time a patient treated with another therapy survives.

By "treating, stabilizing, or preventing cancer" is meant causing a reduction in the size of a tumor, slowing or preventing an increase in the size of a tumor, increasing the disease-free survival time between the disappearance of a tumor and its reappearance, preventing an initial or subsequent occurrence of a tumor, or reducing an adverse symptom associated with a tumor. In one desirable embodiment, the number of cancerous cells surviving the treatment is at least 20, 40, 60, 80, or 100% lower than the initial number of cancerous cells, as measured using any standard assay. Desirably, the decrease in the number of cancerous cells induced by administration of a nucleic acid of the invention (e.g., a nucleic acid with substantial complementarily to a nucleic acid associated with cancer such as an oncogne) is at least 2, 5, 10, 20, or 50-fold greater than the decrease in the number of non-cancerous cells. In yet another desirable embodiment, the number of cancerous cells present after administration of a nucleic acid of the invention is at least 2, 5, 10, 20, or 50-

fold lower than the number of cancerous cells present prior to the administration of the compound or after administration of a buffer control. Desirably, the methods of the present invention result in a decrease of 20, 40, 60, 80, or 100% in the size of a tumor as determined using standard methods. Desirably, at least 20, 40, 60, 80, 90, or 95% of the treated subjects have a complete remission in which all evidence of the cancer disappears. Desirably, the cancer does not reappear or reappears after at least 5, 10, 15, or 20 years.

Exemplary cancers that can be treated, stabilized, or prevented using the above methods include prostate cancers, breast cancers, ovarian cancers, pancreatic cancers, gastric cancers, bladder cancers, salivary gland carcinomas, gastrointestinal cancers, lung cancers, colon cancers, melanomas, brain tumors, leukemias, lymphomas, and carcinomas. Benign tumors may also be treated or prevented using the methods and nucleic acids of the present invention.

By "infection" is meant the invasion by a pathogen (e.g., a bacteria, yeast, or virus). At bacterial infection may be due to gram positive and/or gram negative bacteria. In desirable embodiments, the bacterial infection is due to one or more of the following bacteria: *Chlamydophila pneumoniae, C. psittaci, C. abortus, Chlamydia trachomatis, Simkania negevensis, Parachlamydia acanthamoebae, Pseudomonas aeruginosa, P. alcaligenes, P. chlororaphis, P. fluorescens, P. luteola, P. mendocina, P. monteilii, P. oryzihabitans, P. pertocinogena, P. pseudalcaligenes, P. putida, P. stutzeri, Burkholderia cepacia, Aeromonas hydrophilia, Escherichia coli, Citrobacter freundii, Salmonella typhimurium, S. typhi, S. paratyphi, S. enteritidis, Shigella dysenteriae, S. flexneri, S. sonnei, Enterobacter cloacae, E. aerogenes, Klebsiella pneumoniae, K. oxytoca, Serratia marcescens, Francisella tularensis, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia alcalifaciens, P. rettgeri, P. stuartii, Acinetobacter calcoaceticus, A. haemolyticus, Yersinia enterocolitica, Y. pestis, Y. pseudotuberculosis, Y. intermedia, Bordetella pertussis, B. parapertussis, B. bronchiseptica,*

Haemophilus influenzae, H. parainfluenzae, H. haemolyticus, H. parahaemolyticus, H. ducreyi, Pasteurella multocida, P. haemolytica, Branhamella catarrhalis, Helicobacter pylori, Campylobacter fetus, C. jejuni, C. coli, Borrelia burgdorferi, V. cholerae, V. parahaemolyticus, Legionella pneumophila, Listeria monocytogenes, Neisseria gonorrhea, N. meningitidis, Kingella dentrificans, K. kingae, K. oralis, Moraxella catarrhalis, M. atlantae, M. lacunata, M. nonliquefaciens, M. osloensis, M. phenylpyruvica, Gardnerella vaginalis, Bacteroides fragilis, Bacteroides distasonis, Bacteroides 3452A homology group, Bacteroides vulgatus, B. ovalus, B. thetaiotaomicron, B. uniformis, B. eggerthii, B. splanchnicus, Clostridium difficile, Mycobacterium tuberculosis, M. avium, M. intracellulare, M. leprae, C. diphtheriae, C. ulcerans, C. accolens, C. afermentans, C. amycolatum, C. argentorense, C. auris, C. bovis, C. confusum, C. coyleae, C. durum, C. falsenii, C. glucuronolyticum, C. imitans, C. jeikeium, C. kutscheri, C. kroppenstedtii, C. lipophilum, C. macginleyi, C. matruchoti, C. mucifaciens, C. pilosum, C. propinquum, C. renale, C. riegelii, C. sanguinis, C. singulare, C. striatum, C. sundsvallense, C. thomssenii, C. urealyticum, C. xerosis , Streptococcus pneumoniae, S. agalactiae, S. pyogenes, Enterococcus avium, E. casseliflavus, E. cecorum, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. solitarius, Staphylococcus aureus, S. epidermidis, S. saprophyticus, S. intermedius, S. hyicus, S. haemolyticus, S. hominis, and/or *S. saccharolyticus.* Desirably, a nucleic acid is administered in an amount sufficient to prevent, stabilize, or inhibit the growth of a pathogenic bacteria or to kill the bacteria.

In various embodiments, the viral infection relevant to the methods of the invention is an infection by one or more of the following viruses: West Nile virus (e.g., Samuel, "Host genetic variability and West Nile virus susceptibility," Proc. Natl. Acad. Sci. USA August 21, 2002; Beasley, Virology

296:17-23, 2002), Hepatitis, picornarirus, polio, HIV, coxsacchie, herpes (e.g., zoster, simplex, EBV, or CMV), adenovirus, retrovius, falvi, pox, rhabdovirus, picorna virus (e.g., coxsachie, entero, hoof and mouth, polio, or rhinovirus), St. Louis encephalitis, Epstein-Barr, myxovirus, JC, coxsakievirus B, togavirus, measles, paramyxovirus, echovirus, bunyavirus, cytomegalovirus, varicella-zoster, mumps, equine encephalitis, lymphocytic choriomeningitis, rabies, simian virus 40, polyoma virus, parvovirus, papilloma virus, primate adenovirus, and/or BK.

Other aspects and embodiments of the invention are in the detailed description and claims below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a gel electrophoresis that represents the ability of RNase H (from *E. coli*) to cleave the RNA strand of a duplex between oligomers containing a single α-L-RNA monomer and complementary RNA strand. The hybrid containing compound **34** [5'-d(GTC TCT A($^{\alpha L}$U)G GAC CT)] can be found in lanes marked "1" on the gel, the hybrid containing compound **35** [5'-d(GTC ($^{\alpha L}$U)CT ATG GAC CT)] can be found in lanes marked "2" on the gel, and the hybrid containing compound **36** [5'-d(G($^{\alpha L}$U)C TCT ATG GAC CT)] can be found in lanes marked "3" on the gel. The lanes marked with an asterisk were control experiments in which no RNase was used. The figure shows that the modified oligonucleotides **34-36** support RNase H cleavage when hybridized to the ^{32}P-labelled complementary RNA sequence, with oligomer **34** degraded less efficiently than oligomers **35** and **36**. The RNA sequence is 5'-r(AGG UCC AUA GAG AC). The DNA reference sequence is 5'-d(GTC TCT ATG GAC CT).

Figure 2 shows the reaction scheme for the preparation of compounds **21**, **22**, **23**, **27**, **29** and **31**.

Figure 3 shows the reaction scheme for the preparation of compounds **24, 25, 26, 28, 30** and **32**.

Figure 4 shows the reaction scheme for the preparation of compounds **38, 39, 40, 41, 42, 43,** and **44**.

Figure 5 shows the structure of an exemplary compound of the invention.

Figure 6 shows the structures of selected nucleotide monomers: DNA (T), LNA ($\mathbf{T^L}$), pyrene DNA (**Py**), 2'-OMe-RNA [2'-OMe(T)], abasic LNA, phenyl LNA, and pyrenyl LNA. Other exemplary universal bases and methods for synthesizing them are disclosed in U.S.S.N. 10/235,683 and IB02/03911, which are hereby incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

Nucleobases

The nucleotide monomers forming part of the oligonucleotide of the present invention comprises a moiety B. The substituent B may designate a group which, when the oligomer is complexing with DNA or RNA, is able to interact (e.g., by hydrogen bonding or covalent bonding or electronic interaction) with DNA or RNA, especially nucleobases of DNA or RNA.

Alternatively, the substituent B may designate a group which acts as a label or a reporter, or the substituent B may designate a group (e.g., hydrogen) which is expected to have little or no interactions with DNA or RNA. Thus, the substituent B is desirably selected from hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

In the present context, the terms "nucleobase" covers naturally occurring nucleobases as well as non-naturally occurring nucleobases. It should be clear

to a person skilled in the art that various nucleobases which previously have been considered "nonnaturally occurring" have subsequently been found in nature. Thus, "nucleobase" includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and tautomers thereof. Illustrative examples of nucleobases are adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C^3-C^6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridine, isocytosine, isoguanine, inosine and the "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272. The term "nucleobase" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleobases are adenine, guanine, thymine, cytosine, 5-methylcytosine, and uracil, which are considered as the naturally occurring nucleobases in relation to therapeutic and diagnostic application in humans.

When used herein, the term "DNA intercalator" means a group which can intercalate into a DNA or RNA helix, duplex or triplex. Examples of functional parts of DNA intercalators are acridines, anthracene, quinones, such as anthraquinone, indole, quinoline, isoquinoline, dihydroquinones, anthracyclines, tetracyclines, methylene blue, anthracyclinone, psoralens, coumarins, ethidium-halides, dynemicin, pyrene, metal complexes such as 1,10-phenanthroline-copper, tris(4,7-diphenyl-1,10-phenanthroline)ruthenium-cobalt, enediynes, such as calcheamicin, porphyrins, distamycin, netropcin, viologen, or daunomycin. Especially interesting examples are acridines, quinones, such as anthraquinone, methylene blue, psoralens, coumarins, and ethidium-halides.

Another group of interesting DNA intercalators comprise as the functional part in the helix, duplex or triplex formation, a moiety of 2 to 6

fused aromatic rings. Examples of such fused aromatic rings are naphthyl, anthracenyl, phen-anthrenyl, pyrenyl, chrysenyl, benzanthracenyl, dibenzanthracenyl, benzopyrenyl, pyrenyl. The DNA intercalators of the invention promise to be a so-called universal nucleobase, that is a nucleobase which pairs with any of the natural occurring nucleobases with a similar binding energy.

In the present context, the term "photochemically active groups" covers compounds which are able to undergo chemical reactions upon irradiation with light. Illustrative examples of functional groups hereof are quinones, especially 6-methyl-1,4-naphtoquinone, anthraquinone, naphtoquinone, and 1,4-dimethyl-anthraquinone, diazirines, aromatic azides, benzophenones, psoralens, diazo compounds, and diazirino compounds.

In the present context "thermochemically reactive group" is defined as a functional group which is able to undergo thermochemically-induced covalent bond formation with other groups. Illustrative examples of functional parts thermochemically reactive groups are carboxylic acids, carboxylic acid esters such as activated esters, carboxylic acid halides such as acid fluorides, acid chlorides, acid bromides, and acid iodides, carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides, thiosemicarbazides, aldehydes, ketones, primary alcohols, secondary alcohols, tertiary alcohols, phenols, alkyl halides, thiols, disulfides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, and boronic acid derivatives.

In the present context, the term "chelating group" means a molecule that contains more than one binding site and frequently binds to another molecule, atom or ion through more than one binding site at the same time. Examples of functional parts of chelating groups are iminodiacetic acid, nitrilotriacetic acid, ethylenediamine tetraacetic acid (EDTA), and aminophosphonic acid.

In the present context, the term "reporter group" means a group which is detectable either by itself or as a part of an detection series. Examples of functional parts of reporter groups are biotin, digoxigenin, fluorescent groups (groups which are able to absorb electromagnetic radiation, e.g. light or X-rays, of a certain wavelength, and which subsequently reemits the energy absorbed as radiation of longer wavelength; illustrative examples are dansyl (5-dimethylamino)-1-naphthalenesulfonyl), DOXYL (Noxyl-4,4-dimethyloxazolidine), PROXYL (N-oxyl-2,2,5,5-tetramethyl-pyrrolidine), TEMPO (N-oxyl-2,2,6,6-tetramethylpiperidine), dinitrophenyl, acridines, coumarins, Cy3 and Cy5 (trade-marks for Biological Detection Systems, Inc.), erytrosine, coumaric acid, umbelliferone, texas red, rhodamine, tetra-methyl rhodamine, Rox, 7-nitrobenzo-2-oxa-1-diazole (NBD), pyrene, fluorescein, Europium, Ruthenium, Samarium, and other rare earth metals), radioisotopic labels, chemiluminescence labels (e.g., labels that are detectable via the emission of light during a chemical reaction), spin labels (e.g., a free radical (e.g., substituted organic nitroxides) or other paramagnetic probes (e.g., Cu^{2+} or Mg^{2+}) bound to a biological molecule being detectable by the use of electron spin resonance spectroscopy), enzymes (such as peroxidases, alkaline phosphatases, P-galactosidases, and glycose oxidases), antigens, antibodies, haptens (groups which are able to combine with an antibody but which cannot initiate an immune response by themselves, such as peptides and steroid hormones), and carrier systems for cell membrane penetration such as, fatty acid residues, steroid moieties (cholesterol), vitamin A, vitamin D, vitamin E, folic acid peptides for specific receptors, groups for mediating endocytose, epidermal growth factor (EGF), bradykinin, and platelet derived growth factor (PDGF). Especially, interesting examples are biotin, fluorescein, Texas Red, rhodamine, dinitrophenyl, digoxigenin, Ruthenium, Europium, Cy5, Cy3, etc.

In the present context "ligand" means something which binds. Ligands can comprise functional groups such as: aromatic groups (such as benzene,

pyridine, naphtalene, anthracene, and phenanthrene), heteroaromatic groups (such as thiophene, furan, tetrahydrofuran, pyridine, dioxane, and pyrimidine), carboxylic acids, carboxylic acid esters, carboxylic acid halides, carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides, thiosemicarbazides, aldehydes, ketones, primary alcohols, secondary alcohols, tertiary alcohols, phenols, alkyl halides, thiols, disulfides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, C_1-C_{20} alkyl groups optionally interrupted or terminated with one or more heteroatoms such as, oxygen atoms, nitrogen atoms, and/or sulfur atoms, optionally containing aromatic or mono/polyunsaturated hydrocarbons, polyoxyethylenes such as, polyethylene glycol, oligo/polyamides such as, poly-β-alanine, polyglycine, and polylysine, peptides, oligo/-polysaccharides, oligo/polyphosphates, toxins, antibiotics, cell poisons, steroids, and "affinity ligands", i.e., functional groups or biomolecules that have a specific affinity for sites on particular proteins, antibodies, polysaccharides, oligosaccharides, and other biomolecules.

It will be clear for a person skilled in the art that the above-mentioned specific examples under DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands correspond to the "active/functional" part of the groups in question. For a person skilled in the art it is furthermore clear that DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands are typically represented in the form M-K- where M is the "active/functional" part of the group in question and where K is a spacer through which the "active/functional" part is attached to the 5- or 6-membered ring. Thus, it should be understood that the group B, in the case where B is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, has the form M-K-, where M is the "active/functional" part of the DNA

intercalator, photochemically active group, thermochemically active group, chelating group, reporter group, and ligand, respectively, and where K is an optional spacer comprising 1-50 atoms, desirably 1-30 atoms, and in particular 1-15 atoms, between the 5- or 6-membered ring and the "active/functional" part.

In the present context, the term "spacer" means a thermo-chemically and photochemically non-active distance-making group and is used to join two or more different moieties of the types defined above. Spacers are selected on the basis of a variety of characteristics including their hydrophobicity , hydrophilicity, molecular flexibility and length (e.g. see Hermanson et. al., "Immobilized Affinity Ligand Techniques", Academic Press, San Diego, California (1992), p. 137). Generally, the length of the spacers are less than or about 400, in some applications desirably less than 100 A. The spacer, thus, comprises a chain of carbon atoms optionally interrupted or terminated with one or more heteroatoms, such as oxygen atoms, nitrogen atoms, and/or sulfur atoms. Thus, the spacer K may comprise one or more amide, ester, amino, ether, and/or thioether functionalities, and optionally substituted aromatic or mono/polyunsaturated hydrocarbons, polyoxyethylene such as polyethylene glycol, oligo/polyamides such as, polyalanine, polyglycine, polylysine, and peptides in general, oligosaccharides, oligo/polyphosphates. Moreover the spacer may consist of combined units thereof. The length of the spacer may vary, taking into consideration the desired or necessary positioning and spatial orientation of the "active/functional" part of the group in question in relation to the 5- or 6-membered ring. In particularly interesting embodiments, the spacer includes a chemically cleavable group. Examples of such chemically cleavable groups include disulfide groups cleavable under reductive conditions and peptide fragments cleavable by peptidases. In one embodiment of the present invention, K designates a single bond so that the "active/functional" part of the group in question is attached directly to the 5- or 6-membered ring.

In a desired aspect of the invention, the substituent B in the general formula (I) is selected from the naturally-occurring nucleobases adenine (A), guanine (G), cytosine (C), thymine (T), 5-methylcytosine, and uracil (U).

Internucleoside linkage

The designation "5'- terminal" refers to the position corresponding to the 5' carbon atom of a ribose moiety in a nucleoside, with "3'-terminal" referring to the position corresponding to the 3' carbon atom of a ribose moiety in a nucleoside. In the oligomers of the present invention (formula I), P designates the radical position for an internucleoside linkage to another monomer, or a 5'-terminal group. The first possibility applies when the α-L-RNA in question is not the 5'-terminal "monomer", whereas the latter possibility applies when the α-L-RNA in question is the 5'-terminal "monomer". It should be understood (which also will be clear from the definition of internucleoside linkage and 5'-terminal group further below) that such an internucleoside linkage or 5'-terminal group may include the substituent R^5 (or equally applicable, the substituent R^{5*}) thereby forming a double bond to the group P. Analogously, the first possibility applies where the α-L-RNA in question is not the 3'-terminal "monomer", whereas the latter possibility applies when the α-L-RNA in question is the 3'-terminal "monomer".

In the present context, the term "monomer" may relate to naturally occurring nucleosides, non-naturally occurring nucleosides, LNAs, PNAs, as well as α-L-RNA. Thus, the term "successive monomer" relates to the neighboring monomer in the 5'-terminal direction and the term "preceding monomer" relates to the neighboring monomer in the 3'-terminal direction. Such successive and preceding monomers, seen from the position of an α-L-RNA monomer, may be naturally-occurring or non-naturally-occurring nucleosides, or even further α -L-RNA monomers. Consequently, in the

present context (as can be derived from the definitions above), the term "oligomer" means an oligonucleotide modified by the incorporation of one or more α-L-RNA monomers. Also in the present context, the term "oligonucleotide" which is the same as "oligomer" which is the same as "oligo" means a successive chain of nucleoside monomers connected via internucleoside linkages.

The linkage between two successive monomers in the oligo consist of 2 to 4, desiably 3, groups/atoms selected from $-CH_2-$, -O-, -S-, $-NR^H-$, -(C=O)-, $-(C=NR^H)-$, -(C=S)-, $-Si(R'')_2-$, -SO-, $-S(O)_2-$, $-P(O)_2-$, $-PO(BH_3)-$, -P(O,S)-, $-P(S)_2-$, -PO(R")-, $-PO(OCH_3)-$, and $-PO(NHR^H)-$, where R^H is selected from hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl. Illustrative examples of such linkages are $-CH_2-CH_2-CH_2-$, $-CH_2-CO-CH_2-$, $-CH_2-CHOH-CH_2-$, $-O-CH_2-O-$, $-O-CH_2-CH_2-$, $-O-CH_2-CH=$ (including R^5 when used as a linkage to a successive monomer), $-CH_2-CH_2-O-$, $-NR^H-CH_2-CH_2-$, $-CH_2-CH_2-NR^H-$, $-CH_2-NR^H-CH_2-$, $-O-CH_2-CH_2-NR^H-$, $-NR^H-CO-O-$, $-NR^H-CO-NR^H-$, $-NR^H-CS-NR^H-$, $-NR^H-C(=NR^H)-NR^H-$, $-NR^H-CO-CH_2-NR^H-$, -O-CO-O-, $-O-CO-CH_2-O-$, $-O-CH_2-CO-O-$, $-CH_2-CO-NR^H-$, $-O-CO-NR^H-$, $-NR^H-CO-CH_2-$, $-O-CH_2-CO-NR^H-$, $-O-CH_2-CH_2-NR^H-$, -CH=N-O-, $-CH_2-NR^H-O-$, $-CH_2-O-N=$ (including R^5 when used as a linkage to a successive monomer), $-CH_2-O-NR^H-$, $-CO-NR^H-CH_2-$, $-CH_2-NR^H-O-$, $-CH_2-NR^H-CO-$, $-O-NR^H-CH_2-$, $-O-NR^H-$, $-O-CH_2-S-$, $-S-CH_2-O-$, $-CH_2-CH_2-S-$, $-O-CH_2-CH_2-S-$, $-S-CH_2-CH=$ (including R^5 when used as a linkage to a successive monomer), $-S-CH_2-CH_2-$, $-S-CH_2-CH_2-O-$, $-S-CH_2-CH_2-S-$, $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, $-CH_2-SO_2-CH_2-$, -O-SO-O-, $-O-S(O)_2-O-$, $-O-S(O)_2-CH_2-$, $-O-S(O)_2-NR^H-$, $-NR^H-S(O)_2-CH_2-$, $-O-S(O)_2-CH_2-$, $-O-P(O)_2-O-$, -O-P(O,S)-O-, $-O-P(S)_2-O-$, $-S-P(O)_2-O-$, -S-P(O,S)-O-, $-S-P(S)_2-O-$, $-O-P(O)_2-S-$, -O-P(O,S)-S-, $-O-P(S)_2-S-$, $-S-P(O)_2-S-$, -S-P(O,S)-S-, $-S-P(S)_2-S-$, $-O-PO(R'')-O-$, $-O-PO(OCH_3)-O-$, $-O-PO(OCH_2CH_3)-O-$, $-O-PO(OCH_2CH_2S-R)-O-$, $-O-PO(BH_3)-O-$, $-O-PO(NHR^N)-O-$, $-O-P(O)_2-NR^H-$, $-NR^H-P(O)_2-O-$,

-O-P(O,NR^H)-O-, -CH_2-P(O)$_2$-O-, -O-P(O)$_2$-CH_2-, and -O-Si-(R")$_2$-O-; among which -CH_2-CO-NR^H-, -CH_2-NR^H-O-, -S-CH_2-O-, -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-PO(CH_3)-O-, and -O-PO(NHR^N)-O-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl, are desirable. An especially desirable linkage includes the natural phosphodiester (-O-P(O)$_2$-O-) linkage. Further illustrative examples are given in Mesmaeker et. al., Current Opinion in Structural Biology 1995, 5, 343-355 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443. The left-hand side of the internuceoside linkage is bound to the 5-membered ring as substituent P* at the 3'-position, whereas the right-hand side is bound to the 5'-position of a preceeding monomer.

It is also clear from the above that the group P may also designate a 5'-terminal group in the case where the LNA in question is the 5'-terminal monomer. Examples of such 5'-terminal groups are hydrogen, hydroxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substi-tuted aryloxy, monophosphate, diphosphate, triphosphate, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

In the present description and claims, the terms "monophosphate", "diphosphate", and "triphosphate" mean groups of the formula: -O-P(O)$_2$-O-, -O-P(O)$_2$-O-P(O)$_2$-O-, and O-P(O)$_2$-O-P(O)$_2$-O-P(O)$_2$-O, respectively. In a particularly interesting embodiment, the group P designates a 5'-terminal group selected from monophosphate, diphosphate and triphosphate. The triphosphate variant is especially interesting as a enyme substrate.

Analogously, the group P* may designate a 3'-terminal group in the case where the α-L-RNA in question is the 3'-terminal monomer. Examples of such 3'-terminal groups are hydrogen, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

Optional substitutions

In the present context, e.g., in connection with the terms "alkyl", "alkenyl", and "alkynyl", the term "optionally substituted" means that the group in question may be substituted one or several times, desirably 1-3 times, with group(s) selected from hydroxy (which when bound to an unsaturated carbon atom may be present in the tautomeric keto form), C_{1-6}-alkoxy (i.e., C_{1-6}-alkyl-oxy), C_{2-6}-alkenyloxy, carboxy, oxo (forming a keto or aldehyde functionality), C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino- C_{1-6}-alkylaminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkylcarbonylamino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, sulfanyl, C_{1-6}-alkylthio, and halogen, where any aryl and heteroaryl may be substituted as specifically described below for "optionally substituted aryl and heteroaryl".

Desirably, the substituents are selected from hydroxy, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di pyrazolyl,

pyridinyl, pyrazinyl, pyridazinyl, piperidinyl, coumaryl, furyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzo-oxozolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl.

In the present context for the terms "aryl" and "heteroaryl," the term "optionally substituted" means that the group in question may be substituted one or several times, desirably 1-5 times, in particular 1-3 times) with group(s) selected from hydroxy (which when present in an enol system may be represented in the tau-tomeric keto form), C_{1-6}-alkyl, C_{1-6}-alkoxy, oxo (which may be represented in the tautomeric enol form), carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C_{1-6}- alkyl)amino; carbamoyl, mono- and di(C_{1-6}-alkyl)-aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkylcarbonylamino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, sulfanyl, dihalogen-C_{1-4}-alkyl, trihalogen-C_{1-4}-alkyl, and halogen, where aryl and heteroaryl representing substituents may be substituted 1-3 times with C_{1-4}-alkyl, C_{1-4}-alkoxy, nitro, cyano, amino or halogen. Desirable examples are hydroxy, C_{1-6}-alkyl, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, aryl, amino, mono- and di(C_{1-6}-alkyl)amino, and halogen, wherein aryl may be substituted 1-3 times with C_{1-4}-alkyl, C_{1-4}-alkoxy, nitro, cyano, amino or halogen.

"Halogen" includes fluoro, chloro, bromo, and iodo.

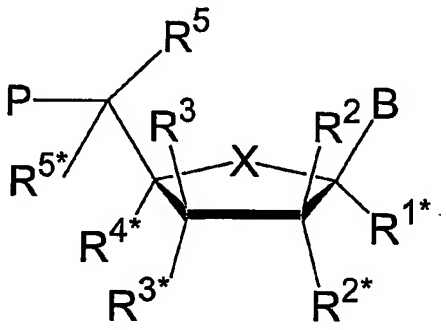
It should be understood that the present invention includes salts (e.g., pharmaceutically acceptable salts) of a-LNA monomers or oligomers that include or or more a-LNA monomers. Salts include acid addition salts and basic salts. Examples of acid addition salts are hydrochloride salts, sodium salts, calcium salts, potassium salts, etc. Examples of basic salts are salts where the (remaining) counter ion is selected from alkali metals, such as sodium and

potassium, alkaline earth metals, such as calcium, and ammonium ions ($^{+}N(R^g)_3R^h$, where each of R^g and R^h independently designates optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl). Pharmaceutically acceptable salts are, e.g., those described in Remington's Pharmaceutical Sciences, 17. Ed. Alfonso R. Gennaro (Ed.), Mack Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in Encyclopedia of Pharmaceutical Technology. Thus, the term "an acid addition salt or a basic salt thereof" used herein is intended to comprise such salts. Furthermore, the oligomers and LNAs as well as any intermediates or starting materials therefor may also be present in hydrate form.

α-L-LNA and β-D-LNA

The oligonucleotide of the present invention may optionally include at least one LNA monomer in addition to the at least one α-L-RNA monomer. The LNA monomer is generally disclosed in WO 99/14226 (the entire content of which is hereby incorporated by reference). The LNA monomer appears in several configurational structures. Particularly, the LNA monomer appears in a α-L-LNA or a β-D-LNA configuration.

While any of the β-D-LNA analogeous disclosed in WO 99/14226 are incorporated herein and may be a part of the the oligo-nucleotide of the invention, desirable β-D-LNA monomers include those of the following formula III



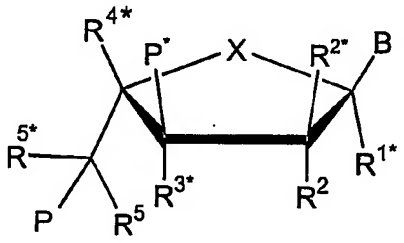
III

where X represent oxygen, sulfur, amino, carbon or substituted carbon, and desirably is oxygen; B is as disclosed for formula I above; R^{1*}, R^2, R^3, R^5 and R^{5*} are hydrogen; P designates the radical position for an inter-nucleoside linkage to another monomer (e.g., a successive monomer) or a 5'-terminal group, R^{3*} is an internucleoside linkage to a another monomer (e.g., a preceeding monomer) or a 3'-terminal group; and R^{2*} and R^{4*} together designate -O-CH_2-, -S-CH_2-, or -NH-CH_2-, where the heteroatom is attached in the 2'-position, or a linkage of -$(CH_2)_n$-, where n is 2, 3 or 4, desirably 2.

β-D-LNA monomers of formula III where R^{2*} and R^{4*} together designates a linkage -O-CH_2- are sometimes referred to as "β-D-oxy-LNA" or, for short, "oxy-LNA"; units of formula III where R^{2*} and R^{4*} contain sulfur are sometimes referred to as "thio-LNA"; and units of formula III where R^{2*} and R^{4*} contain nitrogen are sometimes referred to as "amino-LNA". For many applications, oxy-LNA units are desired modified nucleic acid residues of oligonucleotides of the invention.

The α-L-LNA monomer, which in a desirable embodiment of the invention is present in the oligonucleotide, is disclosed in the international

patent application, publication No. WO 00/66604, the entire content of which is incorporated herein by reference. Desirable α-L-LNA monomers include such having the general formula IV:



IV ,

where X represents oxygen, sulfur, amino, carbon or substituted carbon, and desirably is oxygen; B is as disclosed for formula I above; R^{1*}, R^2, R^{3*}, R^5 and R^{5*} are hydrogen; P designates the radical position for an internucleoside linkage to another monomer (e.g., successive monomer), or a 5'-terminal group; P^* is an internucleoside linkage to another monomer (e.g., precceeding monomer), or a 3'-terminal group; and R^{2*} and R^{4*} together designate $-O-CH_2-$, $-S-CH_2-$, $-NH-CH_2-$, where the hetero atom is attached in the 2'-position, or a linkage of $-(CH_2)_n-$, where n is 2, 3 or 4, desirably 2.

Further examples of useful synthetic nucleotide monomers are *Xylo*-LNA as depicted in WO 00/56748, and L-*ribo*-LNA analogues as depicted in WO 00/66604.

Exemplary Modified Bases such as Universal Bases

Desirable modified bases are covalently linked to the 1'-position of a furanosyl ring, particularly to the 1'-position of a 2',4'-linked furanosyl ring, especially to the 1'-position of a 2'-*O*,4'-C-methylene-beta-D-ribofuranosyl ring.

As discussed above, other desirable modified bases contain one or more carbon alicyclic or carbocyclic aryl units, i.e. non-aromatic or aromatic cyclic

units that contain only carbon atoms as ring members. Modified bases that contain carbocyclic aryl groups are generally desirable, particularly a moiety that contains multiple linked aromatic groups, particularly groups that contain fused rings. That is, optionally substituted polynuclear aromatic groups are especially desirable such as optionally substituted naphthyl, optionally substituted anthracenyl, optionally substituted phenanthrenyl, optionally substituted pyrenyl, optionally substituted chrysenyl, optionally substituted benzanthracenyl, optionally substituted dibenzanthracenyl, optionally substituted benzopyrenyl, with substituted or unsubstituted pyrenyl being particularly desirable.

Without being bound by any theory, it is believed that such carbon alicyclic and/or carbocyclic aryl modified bases can increase hydrophobic interaction with neighboring bases of an oligonucleotide. Those interactions can enhance the stability of a hybridized oligo pair, without necessity of interactions between bases of the distinct oligos of the hybridized pair.

Again without being bound by any theory, it is further believed that such hydrophobic interactions can be particularly favored by platelike stacking of neighboring bases, i.e. intercalation. Such intercalation will be promoted if the base comprises a moiety with a relatively planar extended structure, such as provided by an aromatic group, particularly a carbocyclic aryl group having multiple fused rings. This is indicated by the increases in T_m values exhibited by oligos having LNA units with pyrenyl nucleobases relative to comparable oligos having LNA units with naphthyl nucleobases.

Modified bases that contain one or more heteroalicyclic or heteroaromatic groups also are suitable for use in LNA units, particularly such non-aromatic and aromatic groups that contains one or more N, O or S atoms as ring members, particularly at least one sulfur atom, and from 5 to about 8

ring members. Also desirable is a nucleo base that contains two or more fused rings, where at least one of the rings is a heteroalicyclic or heteroaromatic group containing 1, 2, or 3 N, O, or S atoms as ring members.

In general, desirable are modified bases that contain 2, 3, 4, 5, 6, 7 or 8 fused rings, which may be carbon alicyclic, heteroalicyclic, carbocyclic aryl and/or heteroaromatic; more desirably modified bases that contain 3, 4, 5, or 6 fused rings, which may be carbon alicyclic, heteroalicyclic, carbocyclic aryl and/or heteroaromatic, and desirably the fused rings are each aromatic, particularly carbocyclic aryl.

In some embodiments, the base is not an optionally substituted oxazole, optionally substituted imidazole, or optionally substituted isoxazole modified base.

Other suitable modified bases for use in LNA units in accordance with the invention include optionally substituted pyridyloxazole, optionally substituted pyrenylmethylglycerol, optionally substituted pyrrole, optionally substituted diazole and optionally substituted triazole groups.

Desirable modified bases of the present invention when incorporated into an oligonucleotide containing all LNA units or a mixture of LNA and DNA or RNA units will exhibit substantially constant T_m values upon hybridization with a complementary oligonucleotide, irrespective of the bases present on the complementary oligonucleotide.

In some embodiments, one or more of the common RNA or commonly used derivatives thereof, such as 2'-O-methyl, 2'-fluoro, 2'-allyl, and 2'-O-methoxyethoxy derivatives are combined with at least one nucleotide with a universal base to generate an oligonucleotide having between five to 100 nucleotides.

Modified nucleic acid compounds may comprise a variety of nucleic acid units e.g. nucleoside and/or nucleotide units. As discussed above, an LNA nucleic acid unit has a carbon or hetero alicyclic ring with four to six ring

members, e.g., a furanose ring, or other alicyclic ring structures such as a cyclopentyl, cycloheptyl, tetrahydropyranyl, oxepanyl, tetrahydrothiophenyl, pyrrolidinyl, thianyl, thiepanyl, piperidinyl, and the like.

In an aspect of the invention, at least one ring atom of the carbon or hetero alicyclic group is taken to form a further cyclic linkage to thereby provide a multi-cyclic group. The cyclic linkage may include one or more, typically two atoms, of the carbon or hetero alicyclic group. The cyclic linkage also may include one or more atoms that are substituents, but not ring members, of the carbon or hetero alicyclic group.

Unless indicated otherwise, an alicyclic group as referred to herein is inclusive of group having all carbon ring members as well as groups having one or more hetero atom (e.g. N, O, S or Se) ring members. The disclosure of the group as a "carbon or hetero alicyclic group" further indicates that the alicyclic group may contain all carbon ring members (i.e. a carbon alicyclic) or may contain one or more hetero atom ring members (i.e. a hetero alicyclic). Alicyclic groups are understood not to be aromatic, and typically are fully saturated within the ring (i.e. no endocyclic multiple bonds).

Desirably, the alicyclic ring is a hetero alicyclic, i.e., the alicyclic group has one or more hetero atoms ring members, typically one or two hetero atom ring members such as O, N, S or Se, with oxygen being often desirable.

The one or more cyclic linkages of an alicyclic group may be comprised completely of carbon atoms, or generally more desirable, one or more hetero atoms such as O, S, N or Se, desirably oxygen for at least some embodiments. The cyclic linkage will typically contain one or two or three hetero atoms, more typically one or two hetero atoms in a single cyclic linkage.

The one or more cyclic linkages of a nucleic acid compound of the invention can have a number of alternative configurations and/or configurations. For instance, cyclic linkages of nucleic acid compounds of the invention will include at least one alicyclic ring atom. The cyclic linkage may

be disubstituted to a single alicyclic atom, or two adjacent or non-adjacent alicyclic ring atoms may be included in a cyclic linkage. Still further, a cyclic linkage may include a single alicyclic ring atom, and a further atom that is a substituent but not a ring member of the alicyclic group.

For instance, as discussed above, if the alicyclic group is a furanosyl-type ring, desirable cyclic linkages include the following: C-1', C-2'; C-2', C-3'; C-2', C-4'; or a C-2', C-5' linkage.

A cyclic linkage will typically comprise, in addition to the one or more alicyclic group ring atoms, 2 to 6 atoms in addition to the alicyclic ring members, more typically 3 or 4 atoms in addition to the alicyclic ring member(s).

The alicyclic group atoms that are incorporated into a cyclic linkage are typically carbon atoms, but hetero atoms such as nitrogen of the alicyclic group also may be incorporated into a cyclic linkage.

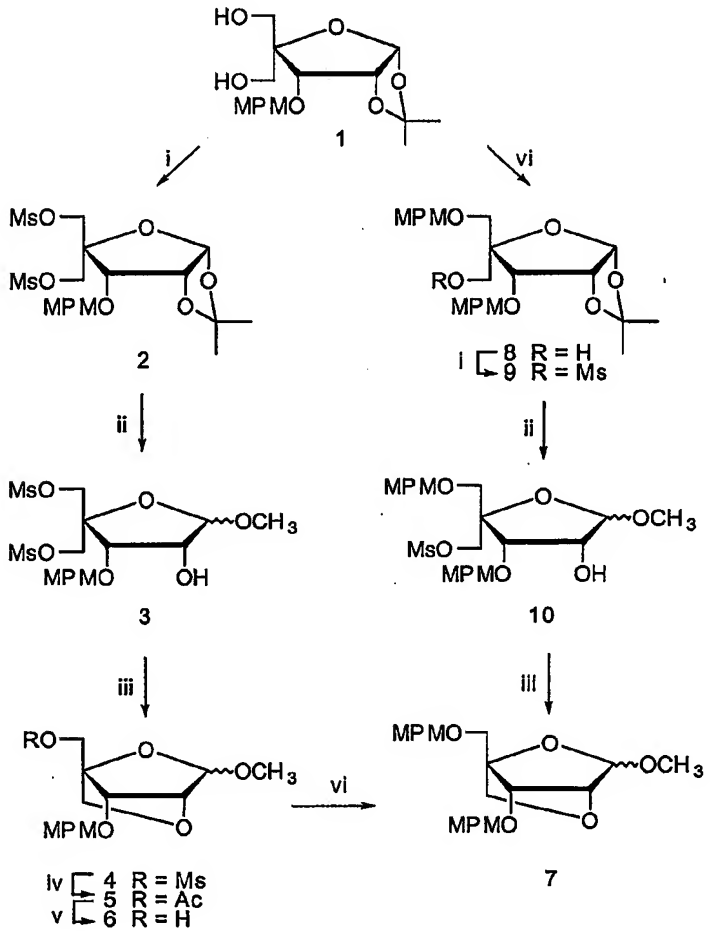
Specifically desirable modified nucleic acids for use oligonucleotides of the invention include locked nucleic acids as disclosed in WO99/14226 (which include bicyclic and tricyclic DNA or RNA having a 2'-4' or 2'-3' sugar linkages); 2'-deoxy-2'-fluoro ribonucleotides; 2'-*O*-methyl ribonucleotides; 2'-*O*-methoxyethyl ribonucleotides; peptide nucleic acids; 5-propynyl pyrimidine ribonucleotides; 7-deazapurine ribonucleotides; 2,6-diaminopurine ribonucleotides; and 2-thio-pyrimidine ribonucleotides.

LNA units as disclosed in WO 99/14226 are in general particularly desirable modified nucleic acids for incorporation into an oligonucleotide of the invention. Additionally, the nucleic acids may be modified at either the 3' and/or 5' end by any type of modification known in the art. For example, either or both ends may be capped with a protecting group, attached to a flexible linking group, attached to a reactive group to aid in attachment to the substrate surface, etc. Desirable LNA units also are disclosed in WO 0056746, WO 0056748, and WO 0066604.

Desirable syntheses of pyrene-LNA monomers is shown in the following Schemes 1 and 2. In the below Schemes 1 and 2, the compound reference numerals are also referred to in the examples below.

A wide variety of modified nucleic acids may be employed, including those that have 2'-modification of hydroxyl, 2′-O-methyl, 2′-fluoro, 2′-trifluoromethyl, 2′-O-(2-methoxyethyl), 2′-O-aminopropyl, 2′-O-dimethyl-amino-oxyethyl, 2′-O-fluoroethyl or 2′-O-propenyl. The nucleic acid may further include a 3' modification, desirably where the 2'- and 3'-position of the ribose group is linked. The nucleic acid also may contain a modification at the 4'-position, desirably where the 2'- and 4'-positions of the ribose group are linked such as by a 2'-4' link of $-CH_2-S-$, $-CH_2-NH-$, or $-CH_2-NMe-$ bridge.

Scheme 1


HO
HO
MPMO
1
i
vi
MsO
MsO
MPMO
2
MPMO
RO
MPMO
I 8 R = H
9 R = Ms
ii
ii
MsO
MsO
MPMO
OH
OCH3
3
MPMO
MsO
MPMO
OH
OCH3
10
iii
iii
RO
MPMO
OCH3
vi
MPMO
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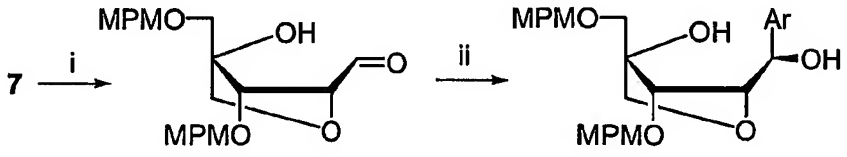
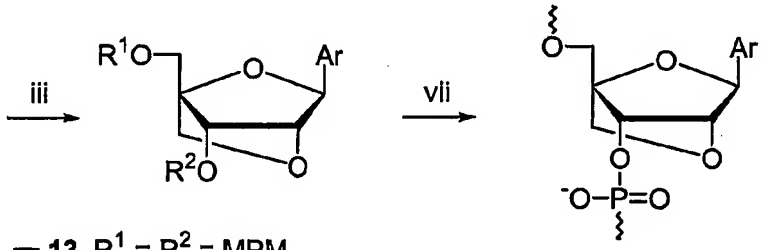
OCH3
IV 4 R = Ms
5 R = Ac
V 6 R = H
7

Scheme 2



11 **12**



17

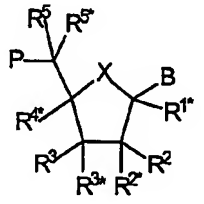
iv **13** $R^1 = R^2 = MPM$
v **14** $R^1 = R^2 = H$
vi **15** $R^1 = DMT$, $R^2 = H$
16 $R^1 = DMT$,
$R^2 = P(O(CH_2)_2CN)N(i\text{-}Pr)_2$

12-17	Ar
a	phenyl
b	4-fluoro-3-methylphenyl
c	1-naphthyl
d	1-pyrenyl
e	2,4,5-trimethylphenyl

The nucleotide also may have a variety of configurations such as α-D-ribo, β-D-xylo, or α-L-xylo configuration.

The internucleoside linkages of the units of oligos of the invention may be natural phosphorodiester linkages, or other linkages such as -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-PO(CH$_3$)-O-, and -O-PO(NHR^N)-O-, where R^H is selected from hydrogen and C$_{1-4}$-alkyl, and R" is selected from C$_{1-6}$-alkyl and phenyl.

A further desirable group of modified nucleic acids for incorporation into oligomers of the invention include those of the following formula:



wherein X is -O-; B is a modified base as discussed above e.g. an optionally substituted carbocyclic aryl such as optionally substituted pyrene or optionally substituted pyrenylmethylglycerol, or an optionally substituted heteroalicylic or optionally substituted heteroaromatic such as optionally substituted pyridyloxazole. Other desirable universal bases include, pyrrole, diazole or triazole moieties, all of which may be optionally substituted. R^{1*} is hydrogen.

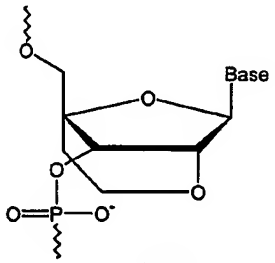
P designates the radical position for an internucleoside linkage to a successive monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R^5, R^5 being hydrogen or included in an internucleoside linkage. R^{3*} is a group P* which designates an internucleoside linkage to a preceding monomer, or a 3'-terminal group. One or two pairs of non-geminal substituents selected from the present substituents of R^2, R^{2*}, R^3, R^{4*}, may designate a biradical consisting of 1-4 groups/atoms selected from $-C(R^aR^b)-$, $-C(R^a)=C(R^a)-$, $-C(R^a)=N-$, -O-, -S-, $-SO_2-$, $-N(R^a)-$, and >C=Z. Z is selected from -O-, -S-, and $-N(R^a)-$, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy,

sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, the possible pair of non-geminal substituents thereby forming a monocyclic entity together with (i) the atoms to which the non-geminal substituents are bound and (ii) any intervening atoms; and each of the substituents R^2, R^{2*}, R^3, R^{4*} which are present and not involved in the possible biradical is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; and basic salts and acid addition salts thereof.

Modified nucleobases and nucleosidic bases may comprise a cyclic unit (e.g. a carbocyclic unit such as pyrenyl) that is joined to a nucleic unit, such as a 1'-position of furasonyl ring through a linker, such as a straight of branched chain alkylene or alkenylene group. Alkylene groups suitably having from 1 (i.e. $-CH_2-$) to about 12 carbon atoms, more typically 1 to about 8 carbon atoms, still more typically 1 to about 6 carbon atoms. Alkenylene groups suitably have one, two or three carbon-carbon double bounds and from 2 to 12 carbon atoms, more typically 2 to 8 carbon atoms, still more typically 2 to 6 carbon atoms.

Exemplary Nucleic Acid Monomers and Oligomers

Desirable LNA units include those that contain a furanosyl-type ring and one or more of the following linkages: C-1', C-2'; C-2', C-3'; C-2', C-4'; or a C-2', C-5' linkage. A C-2', C-4' is particularly desirable. In another aspect of the invention, desirable LNA units are compounds having a substituent on the 2'-position of the central sugar moiety (e.g., ribose or xylose), or derivatives thereof, which favors the C3'-endo conformation, commonly referred to as the North (or simply N for short) conformation. Exemplary LNA units include ENA (2'-O,4'-C-ethylene-bridged nucleic acids such as those disclosed in WO 00/47599 or those illustrated below) units as well as non-bridged riboses such as 2'-F or 2'-O-methyl.



ENA

In various embodiments, the oligonucleotide has at least one LNA unit with a modified base as disclosed herein. Suitable oligonucleotides also may contain natural DNA or RNA units (e.g., nucleotides) with natural bases, as well as LNA units that contain natural bases. Furthermore, the oligonucleotides of the invention also may contain modified DNA or RNA, such as 2'-O-methyl RNA, with natural or modified nucleobases (e.g., pyrene). Desirable oligonucleotides contain at least one of and desirably both of 1) one or more DNA or RNA units (e.g., nucleotides) with natural bases, and 2) one or more LNA units with natural bases, in addition to LNA units with a modified base. In other embodiments, the nucleic acid does not contain a modified base.

Oligonucleotides of the invention desirably contain at least 50 percent or more, more desirably 55, 60, 65, or 70 percent or more of non-modified or natural DNA or RNA units (e.g., nucleotides) or units other than LNA units based on the total number of units or residues of the oligo. A non-modified nucleic acid as referred to herein means that the nucleic acid upon incorporation into a 10-mer oligomer will not increase the T_m of the oligomer in excess of 1°C or 2°C. More desirably, the non-modified nucleic acid unit (e.g., nucleotide) is a substantially or completely "natural" nucleic acid, i.e. containing a non-modified base of uracil, cytosine, 5-methyl-cytosine, thymine, adenine or guanine and a non-modified pentose sugar unit of β-D-ribose (in the case of RNA) or β-D-2-deoxyribose (in the case of DNA).

Oligonucleotides of the invention suitably may contain only a single modified (i.e. LNA) nucleic acid unit, but desirably an oligonucleotide will contain 2, 3, 4 or 5 or more modified nucleic acid units. Typically desirable is where an oligonucleotide contains from about 5 to about 40 or 45 percent modified (LNA) nucleic acid units, based on total units of the oligo, more desirably where the oligonucleotide contains from about 5 or 10 percent to about 20, 25, 30 or 35 percent modified nucleic acid units, based on total units of the oligo.

Typical oligonucleotides that contain one or more LNA units with a modified base as disclosed herein suitably contain from 3 or 4 to about 200 nucleic acid repeat units, with at least one unit being an LNA unit with a modified base, more typically from about 3 or 4 to about 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 or 150 nucleic acid units, with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 LNA units with a modified base being present.

As discussed above, particularly desirable oligonucleotides contain a non- modified DNA or RNA unit at the 3' terminus and a modified DNA or RNA unit at one position upstream from (generally referred to hereing as the –

1 or penultimate position) the 3' terminal non-modified nucleic acid unit. In some embodiments, the modified base is at the 3' terminal position of a nucleic acid primer, such as a primer for the detection of a single nucleotide polymorphism. Other particularly desirable nucleic acids have an LNA unit with or without a modified base in the 5' and/or 3' terminal position.

Also desirable are oligonucleotides that do not have an extended stretches of modified DNA or RNA units, e.g. greater than about 4, 5 or 6 consecutive modified DNA or RNA units. That is, desirably one or more non-modified DNA or RNA will be present after a consecutive stretch of about 3, 4 or 5 modified nucleic acids.

Generally desirable are oligonucleotides that contain a mixture of LNA units that have non-modified or natural nucleobases (i.e., adenine, guanine, cytosine, 5-methyl-cytosine, uracil, or thymine) and LNA units that have modified bases as disclosed herein.

Particularly desirable oligonucleotides of the invention include those where an LNA unit with a modified base is interposed between two LNA units each having non-modified or natural bases (adenine, guanine, cytosine, 5-methyl-cytosine, uracil, or thymine. The LNA "flanking" units with natural base moieties may be directly adjacent to the LNA with modified base moiety, or desirably is within 2, 3, 4 or 5 nucleic acid units of the LNA unit with modified base. Nucleic acid units that may be spaced between an LNA unit with a modified base and an LNA unit with natural nucleobasis suitably are DNA and/or RNA and/or alkyl-modified RNA/DNA units, typically with natural base moieties, although the DNA and or RNA units also may contain modified base moieties.

The oligonucleotides of the present invention are comprised of at least about one universal base. Oligonucleotides of the present can also be comprised, for exmple, of between about one to six 2'-Ome-RNA unit, at least about two LNA units and at least about one LNA pyrene unit.

Exemplary Target Nucleic Acids

In the practice of the present invention, target nuclei acids may be suitably single-stranded or double-stranded RNA; however, single-stranded RNA targets, suchs as mRNAs are desirable. There is substantial guidance in the literature for selecting particular sequences for nucleic acids with LNA or other high affinity nucleotides given a knowledge of the sequence of the target polynucleotide, e.g., Peyman and Ulmann, *Chemical Reviews*, 90:543-584, 1990; Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 32:329-376 (1992); and Zamecnik and Stephenson, *Proc. Natl. Acad. Sci.*, 75:280-284 (1974). Desirable mRNA targets include the 5' cap site, tRNA primer binding site, the initiation codon site, the mRNA donor splice site, and the mRNA acceptor splice site, e.g., Goodchild et al., U.S. Patent 4,806,463.

Intermediates

The synthesis of oligonucleotides may be performed on a automated DNA synthesizer using the phosphoramidite approach. This synthesis method involves the use of nuclosides derivatized on the 3' position of the furanose ring with a phosphoramidite group and on the 5' position of the furanose ring with an acid-labile hydroxy protection group.

The phosphoramidite group has the general formula: -O-P-(NR^8R^{8*})-R^9.

R^8 and R^{8*} may be same or different. Usually, R^8 and R^{8*} are selected from linear and branched optional substituted C_{1-6}-alkyl and C_{1-6}-alkenyl. However, R^8 and R^{8*} also can form alone or together a morpholino group (-$N(CH_2CH_2)_2O$). Desirably, R^8 and R^{8*} represent ethyl, or isopropyl. Allyl is also a possibility.

R^9 is a phosphate protection group. In the original method developed by Caruthers et al (US 4,415,732, US 4,668,777, and US 4,973,679), R^9 is

methoxy. This method has been improved by Köster (US 4,725,677), where a protecting group that can be liberated by β-elimination is suggested. It has also been suggested to use δ-elimination for protecting group removal.

Examples of the phospho protection group R^9 according to the invention are $-O-CH_2-CH_2-CN$, $-S-CH_2-CH_2-CN$, $-O-CH_2-CH=CH_2$, $-S-CH_2-CH=CH_2$, $-O-CH_2-CH=CH-CH_2-CN$, $-S-CH_2-CH=CH-CH_2-CN$, $-O-CH_2-CH=CH-CH=CH_2$, $-S-CH_2-CH=CH-CH=CH_2$, $CCl_3-C(CH_3)_2-O-$, $CCl_3-C(CH_3)_2-S-$, and *p*-nitrophenylethyl.

The phospho protection groups can be removed with suitable means following the synthesis of the oligonucleotide. The specific steps for removal of the protection groups depends largely on the nature of the protection group. Most of the above groups may be removed under appropriate basic conditions. However, the allyloxy groups may require treatment with paladium.

The acid-labile hydroxy protection group of the 5' position of the furanose ring can be selected among a large group of compounds. Illustrative examples of hydroxy protection groups are optionally substituted trityl, such as 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), or trityl, optionally substituted 9-(9-phenyl)xanthenyl (pixyl), optionally substituted ethoxycarbonyloxy, phenylazophenyloxycarbonyloxy, tetrahydropyranyl (THP), 9-fluorenyl-methoxycarbonyl (Fmoc), methoxytetrahydropyranyl (MTHP), silyloxy, such as trimethylsilyl (TMS), triisopropylsilyl (TIPS), tert-butyldimethylsilyl (TBDMS), triethylsilyl, or phenyldimethylsilyl, benzyloxycarbonyl or substituted benzyloxy-carbonyl ethers, such as 2-bromo benzyloxycarbonyl, tert-butylethers, alkyl ethers, such as methyl ether, acetals (including two hydroxy groups), acyloxy such as acetyl, halogen substituted acetyls, such as chloroacetyl or fluoroacetyl, isobutyryl, pivaloyl, benzoyl or substituted benzoyls, methoxymethyl (MOM), and benzyl ethers or substituted benzyl ethers, such as 2,6-dichlorobenzyl (2,6-Cl_2Bn). Presently, DMT is desired as the protection group.

Protection groups

It should be understood that any chemical group (including any nucleobase), which is reactive under the conditions prevailing in chemical oligonucleotide synthesis, is optionally functional-group-protected as known in the art.

This means that groups such as hydroxy, amino, carboxy, sulfono, and mercapto groups, as well as nucleobases, of a monomeric α-L-RNA are optionally functional-group-protected. Protection (and deprotection) is performed by methods known to the person skilled in the art (see, *e.g.*, Greene, T. W. and Wuts, P. G. M., "Protective Groups in Organic Synthe-sis", 2nd ed., John Wiley, N.Y. (1991), and M.J. Gait, Oligonucleotide Synthesis, IRL Press, 1984).

Illustrative examples of hydroxy protection groups are optionally substituted trityl, such as 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), or trityl, optionally substituted 9-(9-phenyl)xanthenyl (pixyl), optionally substituted ethoxycarbonyloxy, phenylazophenyloxycarbonyloxy, tetrahydropyranyl (THP), 9-fluorenylmethoxycarbonyl (Fmoc), methoxytetrahydropyranyl (MTHP), silyloxy, such as trimethylsilyl (TMS), triisopropylsilyl (TIPS), tert-butyldimethylsilyl (TBDMS), triethylsilyl, or phenyldimethylsilyl, benzyloxycarbonyl or substituted benzyloxycarbonyl ethers, such as 2-bromobenzyloxycarbonyl, tert-butylethers, alkyl ethers, such as methyl ether, acetals (including two hydroxy groups), acyloxy, such as acetyl, halogen substituted acetyls, such as chloroacetyl or fluoro-acetyl, isobutyryl, pivaloyl, benzoyl or substituted benzoyls, methoxymethyl (MOM), and benzyl ethers or substituted benzyl ethers, such as 2,6-dichlorobenzyl (2,6-Cl_2Bn). Alternatively, the hydroxy group may be protected by attachment to a solid support optionally through a linker.

Illustrative examples of amino protection groups are Fmoc (fluorenylmethoxycarbonyl), BOC (*tert*-butyloxycarbonyl), trifluoroacetyl, allyloxycarbonyl (alloc or AOC), benzyloxycarbonyl (Z or Cbz), substituted benzyloxycarbonyls, such as 2-chlorobenzyloxycarbonyl (2-ClZ), monomethoxytrityl (MMT), dimethoxytrityl (DMT), phthaloyl, and 9-(9-phenyl)-xanthenyl (pixyl).

Illustrative examples of carboxy protection groups are allyl esters, methyl esters, ethyl esters, 2-cyanoethyl esters, trimethylsilylethyl esters, benzyl esters (OBn), 2-adamantyl esters (O-2-Ada), cyclohexyl esters (OcHex), 1,3-oxazolines, oxazoler, 1,3-oxazolidines, amides, and hydrazides.

Illustrative examples of mercapto protecting groups are trityl (Trt), acetamidomethyl (Acm), trimethylacetamidomethyl (Tacm), 2,4,6-trimethoxybenzyl (Tmob), tert-butylsulfenyl (StBu), 9-fluorenylmethyl (Fm), 3-nitro-2-pyridinesulfenyl (Npys), and 4-methylbenzyl (Meb).

Furthermore, it may be necessary or desirable to protect any nucleobase included in a monomeric α-L-RNA, especially when the monomeric LNA is to be incorporated in an oligomer according to the invention. In the present context, the term "protected nucleobases" means that the nucleobase in question is carrying a protection group selected among the groups which are well-known for a those skilled in the art (see *e.g.* Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223; and E. Uhlmann and A. Peyman, Chem. Rev., 90, 543.). Illustrative examples are benzoyl, isobutyryl, tert-butyl, tert-butyloxycarbonyl, 4-chloro-benzyloxycarbonyl, 9-fluorenylmethyl, 9-fluorenylmethyloxycarbonyl, 4-methoxybenzoyl, 4-methoxytriphenylmethyl, optionally substituted triazolo, p-toluenesulfonyl, optionally substituted sulfonyl, isopropyl, optionally substituted amidines, optionally substituted trityl, phenoxyacetyl, optionally substituted acyl, pixyl,

tetrahydropyranyl, optionally substituted silyl ethers, and 4-methoxybenzyloxycarbonyl. Chapter 1 in "Protocols for oligonucleotide conjugates", Methods in Molecular Biology, Vol 26, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, N.J., and S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223 disclose further suitable examples.

In a desirable embodiment, the group B in a monomeric LNA is selected from nucleobases and protected nucleobases.

Synthesis of intermediates

The synthesis of the novel intermediates of the invention starts from the easy accessible compound L-arabinose. The first step is described earlier in literature. Thus, compound **1** (1-[3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-L-arabino-furanosyl]thymine) was obtained in accordance with the directions of Czernecki, S.; le Diguarher, T. *Synthesis* **1991**, 683. Compound **1** was reacted with methanesulfonyl chloride in anhydrous pyridine to activate the 2'-hydroxy as a methanesulfonate group (compound **2**). Subsequently, compound **2** was treated with aqueous sodium hydroxide, affecting stereochemical inversion of the hydroxy substituent at C-2, presumably through an anhydro nucleoside intermediate, to yield compound **3**. Without isolation of compound **3,** further aqueous sodium hydroxide is added and the temperature is raised to remove the protection group 1,1,3,3-tetraisopropyldisiloxane of the 3'- and 5'-position. Thus, compound **4** is obtained and isolated. Compound **4** is subsequently treated with 4,4'-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine to obtain compound **5**, which has the 5'-hydroxy group protected with DMT. Compound **5** was treated with a combination of imidazole and tert-butyldimethylsilyl chloride (TBDMSCl) in anhydrous pyridine to produce compound **6,** which has the 2'-hydroxy group protected with TBDMS and the

3'-hydroxy group unprotected. Finally, phosphoramidite (compound **7**) was made by treating compound **6** with $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$ in a mixture of anhydrous dichloromethane and *N,N*-diisopropylethylamine.

This exemplified synthetic route is directly applicable for synthesis of other pyrimidine derivatives, e.g. uracil-1-yl, cytosin-1-yl or 5-methylcytosin-1-yl. For the corresponding purin-9-yl derivatives, inversion via anhydro nucleoside formation is precluded. Instead, inversion by reaction of purine derivatives of derivative **2** by, e.g., acetate formation followed by deacetylation, is a viable strategy towards synthesis of derivatives corresponding to structure **3** of the purines. The subsequent steps are expected to be applicable as exemplified in order to obtain phosphoramidite derivatives like **7** of other (properly protected) nucleobases, e.g., uracil-1-yl, cytosin-1-yl. 5-methyl-cytosin-1-yl, guanin-9-yl and adenin-9-yl. This has been exemplified herein by the synthesis of the α-L-LNA adenin-9-yl nucleoside **44**, [13C NMR (CDCl3) d 20.6, 63.5, 70.1, 73.8, 76.8, 81.0, 82.7, 122.7, 123.9, 125.4, 128.1, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9, 129.1, 129.4, 129.8, 129.9, 130.1, 132.9, 133.6, 133.7, 136.2, 136.6, 142.8, 149.6, 149.8, 152.8, 164.9, 166.2, 169.3.], cf. Fig. 4, starting from known derivative **37**. Alternatively, synthetic routes employing L-ribofuranose derivatives without a 2-participation group for nucleoside coupling reactions may be envisioned as obvious for a person skilled in the art of nucleoside synthesis. Accordingly, α-L-ribofuranosyl pyrimidine nucleosides have been obtained by condensation between a 1-thiophenyl L-ribofuranose derivative (A. L. Weis, C. T. Goodhue, K. Shanmuganathan, WO 96/13512). It has also been shown that an approach starting from L-ribose followed by oxazoline formation and further transformations may lead to α-L-ribofuranosyl pyrimidine nucleosides, such as 1-(α-L-ribofuranosyl)uracil **23** and 1-(α-L-ribofuranosyl)cytosine **25** (A. L. Weis, C. T. Goodhue, K. Shanmuganathan, WO 96/13512). We have herein

used a similar, but improved, approach to make 1-(α-L-ribofuranosyl)uracil **23** and 1-(α-L-ribofuranosyl)cytosine **25**. Furthermore, we have synthesized the corresponding (base protected) 2'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-dimethoxytrityl derivatives **29** and **30** and subsequently converted these into the key phosphoramidite derivatives **31** and **32** using procedures similar to procedures known for corresponding transformations of equivalent α-D-ribofuranosyl nucleosides (F. Debart, B. Rayner, G. Degols, J.-L. Imbach, Nucleic Acids Res. 1992, 20, 1193). Phosphoramidites **31** and **32** are useful building blocks for synthesis of α-L-RNA oligomers containing α-L-ribofuranosyl uracil or cytosine nucleotide monomers.

Preparation of oligonucleotides

Linear-, branched-, (M. Grtli and B. S. Sproat, *J. Chem. Soc., Chem. Commun.*, 1995, 495; R. H. E. Hudson and M. J. Damha, *J Am. Chem. Soc.*, 1993, 115, 2119; M. Von Bren, G. V. Petersen, K. Rasmussen, G. Brandenburg, J. Wengel and F. Kirpekar, *Tetrahedron,* 1995, 51, 8491) and circular- (G. Prakash and E. T. Kool, *J. Am. Chem. Soc.*, 1992, 114, 3523) oligonucleotides and polynucleotides of the invention may be produced using the polymerisation techniques of nucleic acid chemistry well known to a person of ordinary skill in the art of organic chemistry. Phosphoramidite chemistry (S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223) was used. Alternatively, other standard chemistry, e.g., H-phosphonate chemistry (F. Seela, K. Wörner and H. Rosemeyer, *Helv. Chim. Acta,I 1994, 77, 883)*, phosphotriester chemistry, or enzymatic synthesis may be used. Generally, standard coupling conditions and the phosphoramidite approach were used, but for some monomers of the invention, longer coupling time, and/or repeated couplings with fresh reagents, and/or use of more concentrated coupling reagents were used. As another possibility, activators more active than 1 H-tetrazole could also be used to

increase the rate of the coupling reaction. An example of such activator is pyridine hydrochloride (V. K. Rajwanshi, A.E. Håkansson, B. M. Dahl, and J. Wengel, *Chem. Commun.* 1999, 1395-1396).

In general, the first step for synthesis of the oligonucleotides of the invention according to the phosphoramidite approach is initiated by attaching the first nucleoside monomer to a solid support at the 3'-position of the sugar moeity. The linkage between the 3'-hydroxy group of the nucleoside and the support is desirably base-labile to make it possible to detach the oligonucleotide under mild alkaline conditions, such as 50:50 aqueous methyl:ammonium hydroxide mixture (AMA), after the formation of the oligonucleotide. The first nucleotide is generally protected on all hydroxy groups, *e.g.*, on the 2'-position (in case of RNA), and 5'-position. The protection of the 5'-position is performed with a acid labile group, such as 4,4'-dimethoxytrityl (DMT). Optionally, any exocyclic amino groups on the nucleobase are also protected, desirably with base-labile acyl groups.

Commercially, several solid supports containing 5'-*O*-DMT- and base protected nucleosides are available. In the syntheses reported herein, the starting nucleoside is dT (deoxyribose thymin-1-yl), which does not need protection of the nucleobase (no exocyclic amino groups present) and does not contain a 2'-hydroxy group to be protected. The dT group is attached to the support through a base-labile ester linkage.

The second step involves the removal of the acid-labile DMT group from the 5'-position of the first nucleoside to form the hydroxy group available for reaction. The most commonly used reagent for this detritylation is a diluted solution of dechloroacetic acid in dichloromethane. However, it is also possible to replace dichloromethane with toluene.

The third step includes the addition of a phosphoramidite derivative and an activator. The activator may be tetrazole, a pyridinium salt, or a similar proton-donating compound. The phosphoramidite derivative comprises the

subsequent monomer protected at the 5'-position with DMT or similar acid labile protection group. The 3'-position of the ribose moeity comprises the phosphoramidite group, which is composed of a phosphine group bound to the 3'-carbon of the ribose moiety through a oxygen, where the phosphine group is further connected to an amino group and a phosphate protecting group. Generally the amino group is mono- or disubstituted with a linear or branched alkyl or alkenyl having 1 to 6 carbon atoms. A desirable amino group is N,N-diisopropylamine. The phosphate-protecting group may be selected in accordance with the specific process. Currently, it is desirable to use β-cyanoethyloxy, δ-cyano-2-butenyloxy, methoxy, or allyloxy. In a desirable embodiment used in the experiments reported herein, the phosphoramidite group is N,N-diisopropylamino-2-cyano-ethyloxyphosphino.

Optionally, the second and the third step may be combined. That is, the addition of the acid, such as trichloroacetic acid, may occur simultaneous with the addition of the phosphoramidite derivative and the activator.

The third step results in a condensation of the phosphoramadite derivative with the first nucleoside attached to the support. The generally accepted mechanism for the condensation involves catalysis (e.g., nucleophilic catalysis) by tetrazole or similar activator for the phosphoramidite coupling reaction. It is believed that a proton is donated by the activator to the phosphorus atom to produce a phosphinium ion. The protonation reaction is followed by nucleophilic displacement of the amino group with tetrazolide or similar activator. Reaction with the 5'-hydroxy group of the support bound first nucleoside generates the dinucleoside phosphite.

Even though a high yield is generally obtained in the third step, unreacted 5'-hydroxy groups may exist. In an optional fourth step, any unreacted hydroxy groups are capped, e.g., with acetyl groups. An acetylation of the 5'-hydroxy groups may be performed by adding acetic anhydride and *N*-methylimidazole.

The fifth step of the process involves oxidation of the phosphite triester resulting from step three to the corresponding phosphodiester. This oxidation can be executed by addition of aqueous iodine or a similar oxidation agent.

If further monomers are to be added to the dinucleotide, steps two through five are repeated. When the oligonucleotide has reached the desired length and order of sequence, the oligonucleotide is released from the support and the phosphorus and nucleobase protecting groups are removed by addition of a mild base, such as aqueous ammonia. The DMT protection group of the 5'-hydroxy group of the last added nucleotide can be removed prior or subsequent to purification using an appropriate acid. The purification of the oligonucleotide is suitably performed on a laboratory scale by reversed-phase HPLC or anion exchange chromatography.

Following the phosphoramidite approach, a stepwise coupling yield of around 98% was afforded starting from the amidite monomer.

An additional aspect of the present invention is to furnish procedures for oligonucleotide analogues containing α-L-RNA linked by non-natural internucleoside linkages. For example, synthesis of the corresponding phosphorothioate or phosphoramidate analogues is possible using strategies well-established in the field of oligonucleotide chemistry (Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223; E. Uhlmann and A. Peyman, *Chem. Rev.*, 90, 543), and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443.

Generally, the present invention also provides the use of an α-L-RNA defined herein for the preparation of α-L-RNA modified oligonucleotides. It should be understood that an α-L-RNA modified oligonucleotide may comprise normal nucleosides (i.e. naturally occurring nucleosides such as ribonucleosides and/or deoxyribonucleosides), as well as modified nucleosides not

occuring in nature. In a particularly interesting embodiment, incorporation of α-L-RNA modulates the ability of the oligonucleotide to act as a substrate for nucleic acid active enzymes.

Furthermore, solid-support materials having immobilized thereto an optionally nucleobase-protected and optionally 5'-hydroxy protected α-L-RNA are especially interesting as material for the synthesis of α-L-RNA modified oligo-nucleotides where an LNA monomer is included at the 3' end. In this instance, the solid support material is CPG or a polymeric material, e.g., polystyrene. In particular, the CPG is a readily (commercially) available CPG material onto which a 3'-functionalized, optionally nucleobase-protected and optionally 5'-hydroxy protected α-L-RNA is linked using the conditions stated by the supplier for that parti-cular material. For example, BioGenex Universial CPG Support (BioGenex, U.S.A.) can be used. The 5'-hydroxy protecting group may be a DMT group. The 3'-functional group can be selected by one skilled in the art with due regard to the conditions applicable for the CPG material in question.

Design of oligonucleotides

The composition of each of the monomers, the order of such monomers, and the total number of monomers of the oligonucleotide of the invention constitute the design of a nucleotide.

The composition of most-desired monomers, and notably the α-L-RNA monomer, is described above. The number, order, and presence of further nucleosides may be of importance in the design of an oligonucleotide intended to hybridize to a specific target. It should be noticed that a successful design of an oligonucleotide of the invention toward a specific target oligonucleotide does not necessarily mean that a like design would be successful toward a different complementary target nucleoside.

The at least one α-L-RNA monomer may, in principle, be placed anywhere in the oligonucleotide, e.g., in the central part, close to or at the 3' end, or close to or at the 5' end. If more than one α-L-RNA monomer is present in the oligonucleotide of the invention, these monomers may appear as single α-L-RNA monomers dispersed throughout the oligonucleotide, or the α-L-RNA monomers can be grouped in blocks, or can appear as mixtures of single and grouped α-L-RNA monomers. In the examples, it is shown that by incorporating a single α-L-RNA monomer in the central part of a 9-mer DNA oligonucleotide, the melting temperature (T_m) decreased toward the DNA complement while the melting temperature was maintained unchanged toward the RNA complement.

In initial studies, it was revealed that oligonucleotides comprising α-L-RNA monomers sometimes showed a lower hybridization tendency compared to the corresponding DNA reference. It may be possible to increase the hybridization power by incorporating LNA monomers into the oligonucleotide. The LNA monomer may have the "normal" β-D configuration or the α-L-configuration. The LNA monomer may occur in the nucleotide, in addition to the α-L-RNA monomer(s), in any appropriate number. Furthermore, the LNA monomer may appear in the oligonucleotide as single entities spread over the entire oligonucleotide or may be present in groups of 2 or more LNA monomers.

Presently, it is desirable to place the one or more LNA adjacent to the one or more α-L-RNA monomer in the oligonucleotide. It is believed that the adverse effect on the affinity shown by the α-L-RNA monomer in some oligomers herein is counteracted by the close presence of the LNA.

When the α-L-RNA monomers and the LNA monomers are present in groups, it is desirable to find not just an increase in the affinity, but also an increase in preferred hybridization to the RNA complement. Most desirable is

when no hybridization with the DNA complement is detected. Moreover, it is desirable that the above effect for oligonucleotides containing blocks of α-L-RNA monomers and LNA monomers are apparent for blocks of α-L-LNA as well as for β-D-LNA.

A similar effect, that yields a high discriminating power and an acceptable affinity, is obtained when single α-L-RNA monomers alternate with single α-L-LNA monomers.

It is noteworthy that the introduction of α-L-RNA monomers in an oligonucleotide produces significant stabilization towards nucleolytic degradation (Example 7).

The ability of RNase H (from *E. coli*) to cleave the RNA strand of a duplex between oligomers containing a single α-L-RNA monomer and complementary RNA strand has been evaluated (Example 8). RNase H efficiently cleaves the duplexes despite the unnatural configuration of the α-L-RNA monomer. It has therefore been shown that it is possible to design α-L-RNA-containing oligomers, such as DNA oligomers, that display the ability of supporting RNase H mediated cleavage when hybridized to RNA.

Applications

The present invention discloses the surprising fact that novel α-L-RNA monomers, when incorporated into oligomers, dramatically increase the discrimination power toward hybridization to a complementary single stranded RNA oligonucleotide. It is possible to design an α-L-RNA modified oligonucleotide which displays an ability to hybridize to the corresponding RNA complement only, while it is not possible to measure any hybridization under the prevailing conditions with the corresponding DNA complement. Furthermore, the oligonucleotides of the invention seem to be unable to be

digested by phosphodiesterases. The above properties of the oligonucleotide of the invention, suggest a variety of applications within the diagnostic and therapeutic field.

In the therapeutic field, the application of an antisense or RNAi; approach is very promising. In short, the antisense approach pertains to the inhibition or destruction of mRNA before it is translated into a peptide or protein. One of the complications with previous oligonucleotides have been that they, besides binding to the mRNA, also bind to DNA in the cell. The binding to genomic DNA may de detrimental for the treated organism because it may alter the expression of the genes at or in the vicinity of the part of the genomic DNA to which the oligonucleotide has hybridized. This unintended hybridization to genomic DNA may be fully or partly avoided by the oligonucleotides of the present invention due to the preferred hybridization to RNA.

Experiments reported herein indicate that the α-L-RNA modified nucleotide of the invention is not, or only very slowly, digested by phosphodiesterases. Thus, a therapeutic agent comprising this oligonucleotide may be present in a biological environment with little or no degradation. This implies that the therapeutic agent, once administered to the organism, may have a long active period in which it can exert the therapeutic affect. It has been demonstrated that oligomers containing one α-L-RNA modified nucleotide have the ability to recruit RNase H. Thus, when the mRNA is captured in the biological environment by hybridization to the α-L-RNA modified nucleotide of the invention, digestion of the captured mRNA is conducted by RNase H. In other words, the oligonucleotide of the invention holds the target mRNA in a vice-like grip, allowing the RNase H to degrade the target mRNA.

An alternative way of effecting cleavage of the RNA target is to use oligomers containing α-L-RNA modified nucleotide(s). DNAzymes function as specific RNA endonucleases by binding to predetermined sequences in an

RNA and cleaving the phosphodiester backbone (R. R. Breaker, G. F. Joyce, *Chem. Biol.* 1994, *1*, 223). Highly efficient, sequence-specific cleavage of RNA is a prerequisite for the use of DNAzymes both as therapeutic antisense oligonucleotides and as general tools for manipulation of RNA. The most commonly used DNAzymes are derivatives of the 31-nucleotide "10-23" oligomer, which was originally isolated by in vitro selection (S. W. Santoro, G. F. Joyce, Proc. *Natl. Acad. Sci. USA* 1997, *94*, 4262). This DNAzyme attains its high specificity through hybridization of its two binding arms to complementary sequences immediately adjacent to the point of cleavage in the RNA substrate. It has recently been shown that incorporation of α-L-LNA nucleotide monomers into the binding arms of a DNAzyme markedly increase the efficiency of RNA cleavage (B. Vester, L. B. Lundberg, M. D. Sørensen, B. R. Babu, S. Douthwaite, J. Wengel, *J. Am. Chem. Soc.* 2002, *124*, ASAP). DNAzymes containing α-L-RNA modified nucleotide(s) should therefore find use because of the RNA selectivety of oligomers containing α-L-RNA modified nucleotide(s) as described above for more traditional antisense oligomers.

The α-L-RNA modified nucleotide may also find application in oligomers designed to strand-invade dsRNA targets. The RNA selective hybridization should make the targeting of both RNA strands possible by applying not only the oligomer containing α-L-RNA modified nucleotide(s) but also an oligomer, *e.g.* a DNA oligomer, not able to hybridize to the oligomer containing α-L-RNA modified nucleotide(s).

The α-L-RNA modified nucleotide may also find application in the combat of RNA-based viruses. It is believed that the oligonucleotide of the invention can selectively bind to viral RNA at various stages of the viral life cycle and therefore hamper the development of the disease caused by the virus.

Other classes of cellular RNAs, e.g., tRNA, rRNA, snRNA, scRNA, which have not previously attracted much attention as targets for the use of antisense or RNAi technology may potentially be regulated by the α-L-RNA modified oligonucleotides of the invention. Furthermore, double stranded RNAs are known to inhibit the growth of several types of cancers and viruses. The α-L-RNA modified oligonucleotides of the invention may have potential applications within these therapeutic fields.

Several diagnostic and molecular biology procedures have been developed that utilize arrays of different oligonucleotides to simultaneously analyse a target nucleic acid, e.g., for the presence of a plethora of possible mutations. Typically, the oligonucleotide arrays are immobilized in a predetermined pattern on a solid support such that the presence of a particular mutation in the target nucleic acid can be revealed by the position on the solid support where it hybridizes. One important prerequisite for the successful use of arrays of different oligonucleotides in the analysis of nucleic acids is that they are all specific for their particular target sequence under the single applied hybridization condition.

Usually, the array of oligonucleotides hybridize to DNA as well as RNA target nucleic acid in a biological sample because the traditional oligonucleotide probes composed of DNA and/or RNA monomers do not substantially discriminate between RNA and DNA. The consequence is that the signal to noise ratio decreases, especially for crude biological samples, which may neccesiate a purification step prior to the sample analysis. The present invention alleviates the problem associated with the higher noise using conventional oligonucleotide probes and provides an oligonucleotide which may be designed with high RNA/DNA discriminating power. Furthermore, using the oligonucleotides of the invention may decrease the need for a purification of the biological sample comprising the target nucleic acid prior to sample testing.

In a desirable embodiment, the acceptable affinity and high specificity toward RNA is exploited in the sequence specific capture and purification of natural or synthetic nucleic acids. In one aspect, the natural RNA or the synthetic RNA analogous are contacted with the α-L-RNA modified oligonucleotide immobilized on a solid surface. In this case hybridization and capture occurs simultaneously. The captured RNA may be, for instance, detected, characterised, quantified, or amplified directly on the surface by a variety of methods well known in the art, or it may be released from the surface before such characterisation or amplification occurs by subjecting the immobilized α-L-RNA modified oligonucleotide and captured nucleic acid to dehybridizing conditions, such as heat or low ionic strength buffers.

The solid support may be chosen from a wide range of polymer materials such as, for example, CPG (controlled pore glass), polypropylene, polystyrene, polycarbonate, or polyethylene and it may take a variety of forms such as, for example a tube, a microtiter plate, a stick, a bead, or a filter. The α-L-RNA modified oligonucleotide may be immobilized to the solid support via its 5' or 3' end (or via the terminus of linkers attached to the 5' or 3' end) by a variety of chemical or photochemical methods usually employed in the immobilization of oligonucleotides or by non-covalent coupling such as for instance via binding of a biotinylated α-L-RNA modified oligonucleotide to immobilized streptavidin. One desirable method for immobilizing α-L-RNA modified oligonucleotides on different solid supports is by photochemical linkage using a photochemically active anthraquinone covalently attached to the 5' or 3' end of the modified oligonucleotide (optionally via linkers) as described in (WO 96/31557). Thus, the present invention also provide a surface carrying an α-L-RNA modified oligonucleotide.

The conventional method for recovery of RNA from a biological sample involves the treatment of the sample with a phenolic solvent to precipitate the

RNA, subsequent centrifugation, and then dissolution of the RNA pellet. This method is time consuming and laborious. The present invention provides the possibility of immobilizing the α-L-RNA modified oligonucleotide on a support in a suitable vessel, like a column, charging the vessel with a biological sample to hybridize the sample RNA to the probes, and subsequently eluting the sample RNA with a suitable buffer. The method may be designed to capture a specific known RNA or a pool of mRNA. The latter possibility is obtained by exploiting the presence of a polyadenine (poly-A) tail of mRNAs produced in all organisms. A probe containing a complementary polythymine (poly-T) stretch can capture the mRNAs having a poly-A tail. Thus, immobilizing α-L-RNA oligonucleotides harbouring a continous base sequence stretch of a plurality of adenine nucleobases provides a potential means for capturing mRNA.

In another aspect, the α-L-RNA modified oligonucleotide carries a ligand covalently attached to either the 5' or 3' end. In this case the α-L-RNA modified oligonucleotide is contacted with the natural or synthetic nucleic acids in solution after which the hybrids formed are captured onto a solid support carrying molecules that can specifically bind the ligand.

In another desirable embodiment, α-L-RNA modified oligonucleotides designed with the purpose of high specificity are used as primers in the sequencing of nucleic acids or as primers in any of the several well known amplification reactions, such as the PCR reaction. The products of the amplification reaction can be analysed by a variety of methods applicable to the analysis of amplification products generated with normal DNA/RNA primers. In the particular case where the α-L-RNA modified oligonucleotide primers are designed to sustain a linear amplification, the resulting amplicons will carry single stranded ends that can be targeted by complementary probes without

denaturation. Such ends could, for example, be used to capture amplicons by other complementary α-L-RNA modified oligonucleotides attached to a solid surface.

In recent years, novel classes of probes have been developed for real-time detection of amplicons generated by target amplification reactions. One such class of probes have been termed "Molecular Beacons". These probes are synthesised as partly self-complementary oligonucleotides containing a fluorophore at one end and a quencher molecule at the other end. When free in solution, the probe folds up into a hairpin structure (guided by the self-complimentary regions) which positions the quencher in sufficient closeness to the fluorophore to quench its fluorescent signal. Upon hybridization to its target nucleic acid, the hairpin opens, thereby separating the fluorophore and quencher and giving off a fluorescent signal.

Another class of probes have been termed "Taqman probes". These probes also contain a fluorophore and a quencher molecule. Contrary to the Molecular Beacons, however, the quencher's ability to quench the fluorescent signal from the fluorophore is maintained after hybridization of the probe to its target sequence. Instead, the fluorescent signal is generated after hybridization by physical detachment of either the quencher or fluorophore from the probe by the action of the 5' exonuclease activity of a polymerase which has initiated synthesis from a primer located 5' to the binding site of the Taqman probe.

High affinity and selectivity for the target site is an important feature in both types of probes and consequently such probes tends to be fairly large (typically 30 to 40 mers). As a result, significant problems are encountered in the production of high quality probes. Therefore, in a desirable embodiment, α-L-RNA monomers in combination with LNA monomers are used to improve production and subsequent performance of Taqman probes and Molecular Beacons by reducing their size whilst retaining the required affinity.

Pharmaceutical composition

The pharmaceutical preparations of the present invention comprise novel oligonucleotides and/or their physiologically tolerated salts in addition to pharmaceutically unobjectionable excipients and/or auxiliary substances. The pharmaceutical preparations of the present invention also include novel nucleoside analogues of the formula II and pharmaceutically acceptable derivatives thereof including physiologically tolerated salts and esters in addition to pharmaceutically unobjectionable excipients and/or auxiliary substances. These analogues may be especially valuable as viral replication inhibitors.

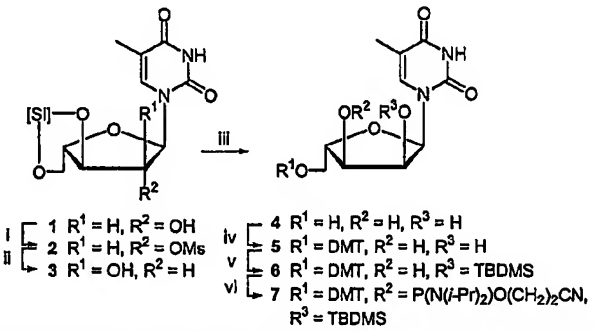
The oligonucleotides and/or their physiologically tolerated salts can be administered to animals, desirably mammals, and in particular humans, as pharmaceuticals on their own, in mixtures with each other, or in the form of pharmaceutical preparations which permit topical, percutaneous, parenteral, or enteral use and which comprise, as the active constituent, an effective dose of at least one oligonucleotide in addition to customary pharmaceutically unobjectionable excipients and auxiliary substances. The preparations normally comprise from about 0.1 to 90% by weight of the therapeutically active compound. In order to treat restenosis, for example, in the form of administration using a catheter, a topical use is desirable. In the case of cancer, infusions and oral administration are desirable. For the treatment of osteoporosis, oral administration is desirable.

The pharmaceutical products are prepared in a manner known per se (e.g., Remingtons Pharmaceutical Sciences, Mack Publ. Co., Easton, PA), with pharmaceutically inert inorganic and/or organic excipients being used. Lactose, corn starch and/or derivatives thereof, talc, stearic acid and/or its salts, etc. can, for example, be used for preparing pills, tablets, coated tablets and hard gelatin capsules. Examples of excipients for soft gelatin capsules and/or suppositories are fats, waxes, semisolid and liquid polyols, natural and/or

hardened oils, etc. Examples of suitable excipients for preparing solutions and/or syrups are water, sucrose, invert sugar, glucose, polyols, etc. Suitable excipients for preparing injection solutions are water, alcohols, glycerol, polyols, vegetable oils, etc. Suitable excipients for microcapsules, implants and/or rods are mixed polymers of glycolic acid and lactic acid. In addition, liposome formulations which are known to those skilled person (N. Weiner, Drug Develop Ind Pharm 15 (1989) 1523; "Liposome Dermatics, Springer Verlag 1992), for example, HVJ Liposomes (Hayashi, Gene Therapy 3 (1996) 878), are suitable. Dermal administration can also be effected, for example, using ionophoretic methods and/or by means of electroporation. Furthermore, use can be made of lipofectins and other carrier systems, for example, those which are used in gene therapy. Systems which can be used to introduce oligonucleotides in a highly efficient manner into eukaryotic cells, or into the nuclei of eukaryotic cells, are particularly suitable.

In addition to the active ingredients and excipients, a pharmaceutical preparation can also comprise additives, such as fillers, extenders, disintegrants, binders, lubricants, wetting agents, stabilizing agents, emulsifiers, preservatives, sweeteners, dyes, flavorings or aromatizing agents, thickeners, diluents, buffering substances, solvents and/or solubilizing agents, agents for achieving a slow release effect, salts for altering the osmotic pressure, coating agents, or antioxidants. They may also comprise two or more different oligonucleotides and/or their physiologically tolerated salts and, furthermore, in addition to at least one oligonucleotide, one or more different therapeutically active ingredients. The dose can vary within wide limits and is to be adjusted to the individual circumstances in each individual case.

The invention relates to a pharmaceutical composition which comprises at least one oligonucleotide according to the invention that can be used for the treatment of diseases which are associated with abnormal cell proliferation, cell migration, cell differentiation, angiogenesis, retinal neurite outgrowth, bone



resorption, phagocytosis, immune response, signal transduction, and the metastasis of neoplastic cells. Such a pharmaceutical composition can be used for the treatment and prevention of cancer and metastasis of cancer, the treatment and prevention of osteoporosis, the treatment of ocular diseases, chronic inflammation, psorasis, restenosis, and in support of wound healing.

EXPERIMENTAL

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Silica gel 60 (particle size 0.040-0.063 mm, Merck) was used for flash column chromatography, with chromatographic fractions containing product pooled and evaporated. δ–Values are in ppm relative to tetramethylsilane as internal standard (^{1}H and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). Coupling constants are given in Hertz (Hz).

The synthesis reported in the following examples may be summarized in the scheme below

Example 1

1-[2-*O*-Methanesulfonyl-3,5-di-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-α-L-arabinofuranosyl]thymine (2).

Starting from L-arabinose, Compound **1** (1-[3,5-di-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-α-L-arabino-furanosyl]thymine)was obtained in accordance with the directions of Czernecki, S.; le Diguarher, T. *Synthesis* **1991**, 683. Compound **1** (6.75 g; 13.48 mmol) was dissolved in anhydrous pyridine (100 ml) and cooled to -40°C. Methane-sulfonyl chloride (2.64 ml; 26,96 mmol) was added, and the solution was stirred for 20 hours at room temperature. The reaction mixture was cooled in an ice bath and the reaction quenched by addition of water (10 ml). The solvent was removed under reduced pressure and the residue was dissolved in water (150 ml) which was extracted with dichloromethane (3 x 70 ml). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (2 x 100 ml) and brine (2 x 100 ml), and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (0-2% CH_3OH in CH_2Cl_2) to give nucleoside **2** as a white solid material (6.59 g; 84%).

R_f (5% CH_3OH in CH_2Cl_2) 0.62; FAB-MS *m/z* 579 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 9.46 (1 H, br s, NH), 7.03 (1 H, d, J = 1.3 hz, H-6, 5.66-5.65 (2 H, m, H-1′ and H-2′), 4.60 (1 H, m, H-3′), 4.40 (1 H, m, H-4′), 3.99-3.97 (2 H, m, H-5′$_a$ and H-5′$_b$), 3.11 (3 H, s, CH_3SO_2), 1.93 (3 H, d, J = 1.0 Hz, CH_3), 1.13-0.91 (28 H, m, $SiCH(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 163.8 (C-4), 150.5 (C-2), 137.6 (C-6), 111.2 (C-5), 90.3 (C-1′), 84.1 (C-2′), 83.7 (C-4′), 73.9 (C-3′), 61.2 (C-5′), 38.0 (CH_3SO_2), 17.3, 17.2, 17.0, 16.8 16.7 ($SiCH(\underline{C}H_3)_2$), 13.3, 13.0, 12.8, 12.4 ($Si\underline{C}H(CH_3)_2$ and CH_3).

Example 2

1-α-L-Ribofuranosylthymine (4).

To at stirred solution of nucleoside **2** (297 mg; 0.51 mmol) in ethanol (6 ml) was added water (1.5 ml) and 2M aqueous sodium hydroxide (1.03 ml). The solution was stirred for 24 hours at room temperature to afford a *ribo*-configurated nucleoside **3** as intermediate. The intermediate **3** was not isolated but treated with further 2 M aqueous sodium hydroxide (2.06 ml). The reaction mixture was stirred at 65 °C overnight. After neutralization with 1 M aqueous hydrochloric acid (to pH 7), the solvent was removed under reduced pressure, the residue was dissolved in dichloro-methane-methanol (1:1), 30 ml silica gel was added and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (10% CH_3OH in CH_2Cl_2) to give nucleoside **4** as a white solid material (78 mg; 59%).

R_f (10% CH_3OH in CH_2Cl_2) 0.10; FAB-MS *m/z* 259 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 11.2 (1 H, br s, NH), 7.47 (1 H, s, H-6), 6.01 (1 H, d, J = 4.4 Hz, H-1′), 5.41 (1 H, d, J = 4.9 Hz, OH-2′), 5.06 (1H, d, J = 4.9, OH-3′), 4.80 (1 H, d, J = 5.6, OH-5′), 4.14 (1 H, dd, J = 4.4 and 8.8 Hz, H-2′), 4.07-4.01 (2 H, m, H-3′ and H-4′), 3.60 (1 H, ddd, J = 2.4, 5.9 and 12.3 Hz, H-5′$_a$), 3.43 (1 H, m, H-5′$_b$), 1.77 (3 H, d, J = 1.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 164.0 (C-4), 150.6 (C-2), 138.6 (C-6), 107.0 (C-5), 85.1 (C-1′), 83.9, 70.5, 70.4 (C-2′, C-3′and C-4′), 61.2 (C-5′), 12.4 (CH_3).

Example 3

1-[5-O-(4,4′-Dimethoxytrityl)-α-L-ribofuranosyl]thymine (5).

To a stirred solution of nucleoside **4** (413 mg; 1.60 mmol) in anhydrous pyridine (15 ml) was added 4,4′-dimethoxytrityl chloride (650 mg; 1.92 mmol). The solution was stirred for 24 hours, additional 4,4′-dimethoxytrityl chloride (271 mg; 0.80 mmol) was added, and after 2 hours, the reaction was quenched with water (40 ml). The mixture was extracted with dichloromethane

(3 x 40 ml) and the combined organic phase was dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (3-6% CH_3OH in CH_2Cl_2 with 0.5% pyridine) to give nucleoside **5** as a white solid material (834 mg; 93%).

R_f (10% CH_3OH in CH_2Cl_2) 0.42; FAB-MS *m/z* 561 $[M+H]^+$ and 560 $[M]^+$; ^1H NMR ($CDCl_3$) δ 10.57 (1 H, br s, NH), 7.46-7.15 (10 H, m, H-6 and ArH), 6.84-6.81 (4 H, m, ArH), 6.25 (1 H, d, $J = 4.6$, H-1′), 5.50 (1 H, br s, OH-2′), 4.75 (1 H, d, $J = 4.7$, H-2′), 4.34 (1 H, dd, $J = 4.2$ and 8.6, H-4′), 4.27 (1 H, m, H-3′), 3.77 (6 H, s, OCH_3), 3.41 (1 H, m, H-5′$_a$), 3.14-3.21 (2 H, m, H-5′$_b$ and OH-3′), 1.85 (3 H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.3 (C-4), 158.5 (C-aryl), 151.3 (C-2), 144.6 (C-aryl), 138.4 (C-6), 135.8, 135.7, 130.0, 129.1, 129.0, 128.2, 128.1, 127.6, 126.8 and 113.2 (aryl-C), 108.6 (C-5), 87.1 and 86.5 (C-1′ and aryl-C), 84.2 (C-4′), 71.9 (C-3′), 71.3 (C-2′), 63.8 (C-5′), 55.2 (OCH_3), 12.4 (CH_3).

Example 4

1-[5-O-(4,4′-Dimethoxytrityl)-2-O-tert-butyldimethylsilyl-α-L-ribofuranosyl]thymine (6).

To a stirred solution of nucleoside **5** (559 mg; 0.997 mmol) in anhydrous pyridine (20 ml) was added imidazole (407 mg; 5.98 mmol) and tert-butyldimethylsilyl chloride (450 mg; 2.99 mmol). The reaction mixture was stirred for 14 hours whereupon saturated aqueous sodium hydrogen carbonate (60 ml) was added. The resulting mixture was extracted with dichloromethane (3 x 60 ml), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure to give a residue which was purified by silica gel column chromatography (2-6% acetone in CH_2Cl_2 with 0.5% pyridine) to give nucleoside **6** as a white solid material (303 mg; 45%).

R_f (5% CH_3OH in CH_2Cl_2) 0.52; FAB-MS *m/z* 675 $[M+H]^+$ and 674 $[M]^+$; ^1H NMR ($CDCl_3$) δ 8.65 (1 H, br s, NH), 7.41-7.17 (10 H, m, 10 H, H-6

and ArH), 6.86-6.83 (4H, m, ArH), 6.47 (1 H, d, J = 5.8 Hz, H-1′), 4.77 (1 H, m, H-2′), 4.32 (1 H, m, H-4′), 4.11 (1 H, m, H-3′), 3.79 (6 H, s, OCH_3), 3.45 (1 H, dd, J = 3.5 and 10 Hz, H-5′$_a$), 3.10 (1H, dd, J = 2.8 and 10.5 Hz, H-5′$_b$), 2.52 (1 H, d, J = 3.5 Hz, OH-3′), 1.92 (3 H, d, J = 1.1 Hz, CH_3), 0.85 (9 H, s, $SiC(CH_3)_3$), 0.12 (3 H, s, $SiCH_3$), 0.03 (3 H, s, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 165.6 (C-4), 158.5 (C-aryl), 150.4 (C-2), 144.3 (C-aryl), 137.6 (C-6), 135.5, 135.3, 129.8, 128.9, 128.1, 127.9, 126.8 and 113.2 (aryl-C), 109.0 (C-5), 86.7 (C-aryl), 85.5 (C-1′), 83.9 (C-4′), 72.5 and 72.4 (C-2′ and C-3′), 63.9 (C-5′), 55.1 (OCH_3), 25.5 ($SiC(\underline{C}H_3)_3$), 17.9 ($Si\underline{C}(CH_3)_3$), 12.4 (CH_3), -5.3 ($SiCH_3$), -5.5 ($SiCH_3$).

Example 5

1-[3-O-(2-Cyanoethoxy(diisopropylamino)phosphino)-5-O-(4,4′-dimethoxytrityl)-2-O-tert-butyldimethylsilyl-α-L-ribofuranosyl]thymine (7).

Compound **6** (375 mg; 0,557 mmol) was co-evaporated twice with anhydrous acetonitrile, dissolved in anhydrous dichloromethane (3 ml) and *N,N*-diisopropylethylamine (1.5 ml) was added, followed by addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.37 ml; 1.67 mmol). The reaction mixture was stirred for 14 h and then partitioned between dichloromethane (20 ml) and water (20 ml). The organic phase was washed with saturated aqueous sodium hydrogen carbonate (2 x 20 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Purification of the residue by silica gel chromatography (EtOAc:n-hexane:Et_3N, 49:50:1) gave phosphoramidite **7** as a white solid material (357 mg; 73%).

R_f (5% CH_3OH in CH_2Cl_2) 0.47; FAB-MS *m/z* 875 $[M+H]^+$ and 897 $[M+Na]^+$; ^{31}P NMR ($CDCl_3$) δ 151.9 and 151.7.

Example 5A

Oxazoline (21).

A mixture of L-ribose (2.00 g, 13.3 mmol), cyanamide (0.67 g, 16.0 mmol) and powdered potassium carbonate (0.07 g, 0.05 mmol) was stirred at 90 °C for 1 h in anhydrous DMF (15 mL). After cooling to room temperature, the mixture was evaporated under reduced pressure to half volumen and the resulting solution was stored for 20 h at 5 °C. The resulting precipitate was filtered off and recrystallized from 96% aqueous EtOH to give 1.90 g of oxazoline **21** (82%). ^{1}H NMR (DMSO-d_6) δ 3.25-3.42 (m, 2H, H5', H5"), 3.63-3.74 (m, 2H, H3', H4'), 4.56-4.59 (m, 2H, H2', OH), 5.17 (bs, 1H, OH), 5.58 (d, 1H, H1', *J*=4.8 Hz), 6.26 (bs, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 60.4, 71.2, 77.8, 80.8, 98.3, 163.8. MALDI-MS (*m/z*) 197 $[M+Na]^+$.

Example 5B

2-*O*,2'-*O*-Anhydro-1-(α-L-ribofuranosyl)uracil (22).

A mixture oxazoline **21** (1.00 g, 5.75 mmol) in 96% aqueous EtOH (10 mL) and methyl propiolate (1.69 g, 20.11 mmol) was heated under reflux for 2 h. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure and then co-evaporated (under reduced pressure) several times with 96% aqueous EtOH to give 1.05 g of nucleoside **22** (81%) after recrystallization from 96% aqueous EtOH. ^{1}H NMR (DMSO-d_6) δ 3.42-3.72 (m, 3H, H4', H5', H5"), 4.02-4.09 (m, 1H, H3'), 4.86 (t, 1H, OH5', J=5.0 Hz), 5.23 (t, 1H, H2', J= 5.2 Hz), 5.74 (d, 1H, OH3', J=6.9 Hz), 5.88 (d, 1H, H5, J=7.4 Hz), 6.20 (d, 1H, H1', J=5.2 Hz), 7.85 (d,1H, H6, J=7.4 Hz). ^{13}C NMR (DMSO-d_6) δ 59.5, 69.8, 80.7, 81.4, 88.6, 108.8, 136.8, 160.7, 171.0. MALDI-MS (*m/z*) 249 $[M+Na]^+$.

Example 5C

1-(α-L-Ribofuranosyl)uracil (23).

A solution of nucleoside 22 (2.17 g, 9.60 mmol) in 0.2 N aqueous hydrochloric acid (10 mL) was refluxed for 1 h. After cooling to room temperature, the solution was neutralized using Amberlyst IRA 410 [OH^-]. The resin was filtered off and washed with approximately 40 °C warm H_2O. The combined filtrates were evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using AcOEt/MeOH (85:15, v/v) to afford 1.80 g (77%) of nucoeoside **23**. ^1H NMR (DMSO-d_6) δ 3.40-3.61 (m, 2H, H5', H5"), 4.00-4.17 (m, 3H, H2', H3', H4'), 4.82 (bs, 1H, OH), 5.50 (bs, 2H, 2 OH), 5.56 (d, 1H, H5, J=8.3 Hz), 6.01 (d, 1H, H1', J=4.7 Hz), 7.61 (d,1H, H6, J=8.1 Hz). ^{13}C NMR (DMSO-d_6) δ 61.2, 70.3, 70.4, 84.0, 85.1, 99.8, 142.8, 150.7, 163.5. MALDI-MS (*m/z*) 267 $[M+Na]^+$.

Example 5D

1-(2,3,5-Tri-*O*-acetyl-α-L-ribofuranosyl)uracil (24).

Acetic anhydride (2.32 mL, 24.5 mmol) was added to a solution of nucleoside **23** (1.71 g, 7.00 mmol) in anhydrous pyridine (10 mL). The reaction mixture was stirred at room temperature for 12 h. After addition of methanol (5 mL) and stirring for additional 10 min, the mixture was evaporated under reduced pressure to near dryness. The residue was disolved in ethyl acetate (20 mL) and washing was performed using first a saturated aqueous solution of sodium hydrogencarbonate (15 mL) and then brine (15 mL). The separated organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (0-5%, v/v) in chloroform as eluent to afford 2.16 g of nucleoside **24** (83%) as a white powder.

Example 5E

1-(α-L-Ribofuranosyl)cytosine (25).

The Lawesson reagent (1.80 g, 4.45 mmol) was added to a stirred solution of compound **24** (2.06 g, 5.57 mmol) in anhydrous 1,2-dichloroethane (50 mL). The reaction mixture was heated under reflux for 4 h and then cooled to room temperature. Methanol (20 mL) was added and the mixture was evaporated to dryness under reduced presuure to give a residue (the crude thiouracile derivative). This residue was immediately disolved in a saturated solution of ammonia in methanol (100 mL) and the resulting mixture was heated at 100 °C for 3 h in an autoclave. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (5-10%, v/v) in AcOEt as eluent to afford 1.00 g of compound **25** (74%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 3.40-3.63 (m, 2H, H5', H5''), 3.96-4.04 (m, 3H, H2', H3', H4'), 4.77 (bs, 1H, OH), 4.98 (d, 1H, OH, J=5.6 Hz), 5.27 (d, 1H, OH, J=3.5 Hz), 5.66 (d, 1H, H5, J=7.4 Hz), 6.01 (d, 1H, H1', J=2.2 Hz), 7.04 (bd, 2H, NH_2), 7.61 (d,1H, H6, J=8.1 Hz). ^{13}C NMR (DMSO-d_6) δ 61.1, 70.1, 70.6, 83.1, 85.6, 92.2, 143.1, 155.2, 165.5. MALDI-MS (*m/z*) 266 $[M+Na]^+$.

Example 5F

4-*N*-Benzoyl-1-(α-L-ribofuranosyl)cytosine (26).

To a stirred solution of nucleoside 25 (1.00 g, 4.11 mmol) in anhydrous pyridine (20 mL) at 0 °C was added trimethylchlorosilane (3.13 mL, 24.67 mmol). The mixture was stirred at room temperature for 1 h whereupon benzoyl chloride (2.38 mL, 20.56 mmol) was added. After stirring for 5h and cooling of the mixture to 0 °C, H_2O (10 mL) was added and stirring was continued for 5 min. Aqueous ammonia (10 mL, 29%, w/w) was added and the mixture was stirred at room temperature for 15 min. The mixture was then

evaporated to dryness under reduced pressure and coevaporated under reduced presuure with toluene. The residue was purified by column chromatography using a stepwise gradient of methanol (0-10%, v/v) in AcOEt as eluent to afford 1.10 g of compound **26** (77%) as a white powder. Recrystallisation from absolute EtOH provided white crystals.

Example 5G

1-(5-*O*-(4,4'-Dimethoxytrityl)-α-L-ribofuranosyl)uracil (27).

4,4'-Dimethoxytrityl chloride (0.43 g, 1.3 mmol) was added to a solution of compound **23** (0.26 g, 1.07 mmol) in anhydrous pyridine (5 mL). The reaction mixture was stirred at room temperature for 12 h whereupon methanol was added (2 mL). After stirring for additional 10 min, the mixture was poured into a saturated aqueous solution of sodium hydrogencarbonate (25 mL). Extraction was performed using chloroform (3 x 20 mL), and the combined organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (0-8%, v/v) in chloroform as eluent to afford 0.55 g of nucleoside **27** (95%) as a white foam. ^{1}H NMR ($CDCl_3$) δ 3.10-3.39 (m, 2H, H5', H5''), 3.78 (s, 6H, DMT), 4.26-4.29 (m, 1H, H3'), 4.37-4.38 (m, 1H, H4'), 4.64 (t, 1H, H2', J=5.2 Hz), 5.49 (bs, 2H, 2 x OH), 5.65 (d, 1H, H5, J=7.8 Hz), 6.29 (d, H1', J=5.2 Hz), 6.81-7.44 (m, 13H, DMT), 7.68 (d, 1H, H6, J=8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 164.3, 158.4, 150.9, 144.6, 142.7, 135.8, 135.7, 130.0, 129.9, 128.1, 127.8, 126.8, 113.2, 100.4, 86.6, 86.3, 84.3, 72.0, 71.2, 63.3, 52.9. MALDI-MS (*m/z*) 569 $[M+Na]^+$.

Example 5H

4-*N*-Benzoyl-1-(5-*O*-(4,4'-dimethoxytrityl)-α-L-ribofuranosylcytosine (28).

4,4'-Dimethoxytrityl chloride (O.33 g, 1.0 mmol) was added to a solution of compound **26** (0.12 g, 0.49 mmol) in anhydrous pyridine (5 mL). The reaction mixture was stirred at room temperature for 12 h whereupon methanol was added (2 mL). After stirring for additional 10 min, the mixture was poured into a saturated aqueous solution of sodium hydrogencarbonate (25 mL). Extraction was performed using chloroform (3 x 20 mL), and the combined organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (0-8%, v/v) in chloroform as eluent to to afford 0.18 g of nucleoside **28** (95%) as a white foam. ^{1}H NMR ($CDCl_3$) δ 1.39 (1H, OH), 3.17-3.51 (m, 3H, OH, H5', H5"), 3.78 (s, 6H, DMT), 4.28-4.36 (m, 2H, H3', H4'), 4.79 (t, 1H, H2', J=4.3 Hz), 5.49 (bs, 2H, 2 x OH), 6.33 (d, H1', J=4.5 Hz), 6.82-7.89 (m, 20H, DMT, H5, NH), 8.03 (d, 1H, H6, J=7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 55.3, 63.8, 71.3, 72.1, 84.5, 86.5, 88.9, 96.3, 113.3, 127.0, 127.8, 128.0, 128.2, 129.1, 130.2, 133.0, 133.2, 135.8, 136.0, 144.7, 146.5, 156.2, 158.6, 162.5, 166.7. MALDI-MS (*m/z*) 569 $[M+Na]^+$.

Example 5I

1-(2-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4'-dimethoxytrityl)-α-L-ribofuranosyl)uracil (29).

The 5'-*O*-(4,4'-dimethoxytrityl)-α-L-ribonucleoside 27 (3.20 g, 5.90 mmol) and imidazole (1.04 g, 15.2 mmol) were disolved in anhydrous pyridine (60 mL). *tert*-Butyldimethylsilyl chloride (1.15 g, 7.6 mmol) was added and the solution was stirred at room temperature for 24 h. The reaction mixture was then poured into a saturated aqueous solution of sodium hydrogencarbonate

(120 mL) and extraction was performed using chloroform (3 x 80 mL). The combined organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of acetone (0-10%, v/v) in CH_2Cl_2 as eluent to give 1.70 g of the 2'-*O*-*tert*-butyldimethylsilyl-5'-*O*-dimethoxy)trityl derivative 29 (the polarity of the 2'-*O*-*tert*-butyldimethylsilyl isomer **29** was lower than the polarity of the corresponding 3'-*O*-*tert*-butyldimethylsilyl isomer) (44%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ -0.07, 0.00 (2s, 6H, TBDMS), 0.72 (s, 9H, TDDMS), 2.46 (d, 1H, OH, J=2.6 Hz), 2.92-3.39 (m, 2H, H5', H5"), 3.66 (s, 6H, DMT), 3.92-3.95 (m, 1H, H3'), 4.22-4.23 (m, 1H, H4'), 4.72 (t, 1H, H2', J=5.6 Hz), 5.56 (d, 1H, H5, J=8.1 Hz), 6.36 (d, H1', J=6.0 Hz), 6.70-7.28 (m, 13H, DMT), 8.47 (d, 1H, H6, J=8.2 Hz), 8.70 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$) δ -5.3, -5.2, 18.1, 25.7, 55.3, 64.2, 72.7, 72.8, 84.5, 85.9, 87.0, 101.0, 113.3, 113.4, 123.8, 127.1, 128.0, 128.1, 130.0, 135.4, 135.7, 142.0, 144.5, 150.6, 158.7, 163.3. ESI-HRMS (*m/z*) 683.2723 $[M+Na]^+$ (calculated 683.2759).

Example 5J

4-*N*-Benzoyl-1-(2-*O*-*tert*-butyldimethylsilyl-5-*O*-(4,4'-dimethoxytrityl)-α-L-ribofuranosyl)cytosine (30).

The 5'-*O*-(4,4'-dimethoxytrityl)-α-L-ribonucleoside **28** (0.44 g, 0.81 mmol) and imidazole (0.14 g, 2.10 mmol) were disolved in anhydrous pyridine (10 mL). *tert*-Butyldimethylsilyl chloride (0.19 g, 1.05 mmol) was added and the resulting mixture was stirred at room temperature for 24 h. The reaction mixture was then poured into a saturated aqueous solution of sodium hydrogencarbonate (25 mL) and extraction was performed using chloroform (3 x 20 mL). The combined organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of acetone

(0-10%, v/v) in CH_2Cl_2 as eluent to give 0.30 g of nucleoside **30** (the polarity of the 2'-*O-tert*-butyldimethylsilyl isomer **30** was lower than the polarity of the corresponding 3'-*O-tert*-butyldimethylsilyl isomer) (49%). ^{1}H NMR ($CDCl_3$) δ 0.01, 0.15 (2s, 6H, TBDMS), 0.83 (s, 9H, TDDMS), 2.47 (d, 1H, OH3', J=4.3 Hz), 3.12-3.54 (m, 2H, H5', H5''), 3.80 (s, 6H, DMT), 4.17-4.21 (m, 1H, H3'), 4.36-4.38 (m, 1H, H4'), 4.87 (t, 1H, H2', J=5.0 Hz), 6.58 (d, H1', J=5.3 Hz), 6.83-8.01 (m, 20H, DMT, Bz, H5, H6), 8.65 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$) δ -5.3, -5.1, 18.2, 25.9, 55.4, 64.0, 72.7, 73.0, 84.4, 86.9, 87.3, 95.5, 113.4, 127.1, 128.2, 129.2, 130.1, 133.3, 135.6, 135.8, 144.6, 146.5, 158.7, 162.1. ESI-MS (*m/z*) 787 $[M+Na]^+$.

Example 5K

1-(2-*O-tert*-Butyldimethylsilyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphinoxy]-5-*O*-(4,4'-dimethoxytrityl)-α-L-ribofuranosyl)uracil (31).

To a stirred solution of nucleoside **29** (0.26 g, 0.40 mmol) in dichloromethane (10 mL) at room temperature was added *N,N*-diisopropylethylamine (0.69 mL, 3.95 mmol). After dropwise addition of 2-cyanoethyl *N,N'*-diisopropylphosphoramidochloridite (0.38 mL, 1.98 mmol), the mixture was stirred at room temperature for 15 h. Dichloromethane (20 mL) and triethylamine (5 mL) were added and the mixture was washed with saturated aqueous sodium hydrogencarbonate (25 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using *n*-hexane/AcOEt/NEt_3 (50:49.5:0.5, v/v/v) as eluent to afford 0.24 g of the product phosphoramidite **31** as a white foam (70%). ^{31}P NMR (DMSO-d_6) δ 149.9, 151.0. ESI-MS (*m/z*) 883 $[M+Na]^+$.

Example 5L

4-*N*-Benzoyl-1-(2-*O*-*tert*-butyldimethylsilyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphinoxy]-5-*O*-(4,4'-dimethoxytrityl)-α-L-ribofuranosyl)cytosine (32).

To a stirred solution of nucleoside **30** (0.07 g, 0.09 mmol) in dichloromethane (4 mL) at room temperature was added *N,N*-diisopropylethylamine (0.16 mL, 0.92 mmol). After dropwise addition of 2-cyanoethyl *N,N'*-diisopropylphosphoramidochloridite (0.09 mL, 0.46 mmol), the mixture was stirred at room temperature for 15 h. Dichloromethane (5 mL) and triethylamine (1 mL) were added and the mixture was washed with saturated aqueous sodium hydrogencarbonate (5 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using *n*-hexane/AcOEt/NEt_3 (50:49.5:0.5, v/v/v) as eluent to afford 0.05 g of the product phosphoramidite **32** (52%) as a white foam. ^{31}P NMR (DMSO-d_6) δ 150.9, 151.4.

Example 5M

3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-α-L-arabinofuranose (38).

To a solution of compound **37**, cf. Fig. 4, (O. Dahlman, P. J. Garegg, H. Mayer, S. Schramek, Acta Chemica Scandinavia 1986, B40, 15) (7.00 g, 16.36 mmol) in anhydrous DMF (50 mL) at 0 °C was added sodium hydride (1.31 g of a 60% suspension in mineral oil, 32.71 mmol) and benzyl bromide (3.89 mL, 32.71 mmol). The mixture was stirred at room temperature for 5 h and was then evaporated to dryness under reduced pressure. The residue was dissolved in diethyl ether (100 mL) and washing was performed using a saturated aqueous solution of sodium hydrogencarbonate (100 mL) and brine (100 mL). The separated organic phase was dried over anhydrous sodium

sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of ethyl acetate (0-5%, v/v) in petroleum ether as eluent to afford 7.20 g of furanose **38**, cf. Fig. 4, (86%) as a clear oil. ^{13}C NMR ($CDCl_3$) δ 19.2, 26.1, 26.8, 26.9, 63.4, 71.6, 82.8, 85.1, 85.2, 105.7, 112.4, 127.6, 127.7, 127.8, 128.5, 129.7, 133.1, 135.5, 135.6, 137.5.

Example 5N

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-L-arabinofuranose (39).

To a solution of furanose **38** (9.84 g, 19.00 mmol) in THF (150 mL) at room temperature was added a solution of 1 M TBAF (38.0 mL, 38.0 mmol). The mixture was stirred at room temperature for 12 h and was then evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 mL) and washing was performed using brine (2 x 100 mL). The separated organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (0-5%, v/v) in chloroform as eluent to afford 4.20 g of furanose **39**, cf. Fig. 4, (80%) as a clear oil. ^{13}C NMR ($CDCl_3$) δ 26.3, 27.1, 62.7, 71.8, 82.7, 85.2, 85.5, 105.5, 112.9, 127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 137.1.

Example 5O

5-*O*-Benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-L-arabinofuranose (40).

To a solution of derivative **39** (2.60 g, 9.29 mmol) in anhydrous pyridine (10 mL) at 0°C was dropwise added benzoyl chloride (1.62 mL, 13.93 mmol). The mixture was stirred at room temperature for 2 h and was then evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washing was performed using a saturated aqueous solution of sodium hydrogencarbonate (80 mL) and brine (80 mL). The separated organic

phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure and then coevaporated with toluene. The residue was purified by column chromatography using a stepwise gradient of ethyl acetate (0-20%, v/v) in petroleum ether as eluent to afford 2.60 g of **40**, cf. Fig. 4, (74%) as a clear oil. ^{13}C NMR ($CDCl_3$) δ 26.3, 27.1, 64.3, 71.2, 82.2, 82.7, 84.8, 105.8, 113.1, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 129.7, 133.1, 136.9, 166.1.

Example 5P

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-*O*-benzyl-α,β-L-arabinofuranose (**41**, Fig. 4).

A solution of derivative **40** (2.21 g, 5.76 mmol) and sulfuric acid (0.02 mL) in aqueous 80% acetic acid (20 mL) was stirred for 1 h at 50 °C. The mixture was allowed to cool to room temperature and was then concentrated under reduced pressure to a volume of approximately 10 mL whereupon pyridine was added (29 mL). Acetic anhydride (1.63 mL, 17.27 mmol) was added dropwise under stirring and the resulting mixture was heated to 50 °C. After stirring for 6 h, the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washing was performed using a saturated aqueous solution of sodium hydrogencarbonate (40 mL) and brine (40 mL). The separated organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure and then coevaporated with toluene. The residue was purified by column chromatography using a stepwise gradient of ethyl acetate (0-20%, v/v) in petroleum ether as eluent to afford 1.80 g of furanose **41** (mixture of anomers, 73%) as a clear oil. ^{13}C NMR ($CDCl_3$) δ 20.4, 20.7, 20.9, 21.1, 63.4, 64.3, 72.4, 72.7, 77.0, 79.5, 79.6, 80.6, 82.9, 83.1, 93.9, 99.9, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 129.6, 129.7, 129.8, 133.2, 137.1, 137.2, 166.0, 166.1, 169.2, 169.5, 169.6.

Example 5Q

9-(2-*O*-Acetyl-5-*O*-benzoyl-3-*O*-benzyl-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (42, Fig. 4).

Compound **41** (0.70 g, 1.64 mmol) was dissolved in anhydrous acetonitrile (6 mL). 6-*N*-Benzoyladenine (0.59 g, 2.45 mmol) and $SnCl_4$ (0.39 mL, 3.27 mmol) were added, and the resulting mixture was stirred at room temperature for 4 h. A saturated aqueous solution of sodium hydrogencarbonate was added until the evolution of carbon dioxide ceased whereupon the mixture was filtered through a layer of Celite 545. The layer of Celite 545 was washed with chloroform (2 x 50 mL) and the combined filtrate was washed successively with a saturated aqueous solution of sodium hydrogencarbonate (3 x 100 mL) and brine (2 x 100 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using a stepwise gradient of methanol (0-5%, v/v) in chloroform as eluent to afford 0.87 g of nucleoside **42** (88%) as a white solid material. ^{13}C NMR ($CDCl_3$) δ 20.9, 63.5, 72.7, 80.5, 83.7, 84.3, 88.5, 123.1, 128.0, 128.6, 128.7, 129.0, 129.5, 129.7, 129.9, 132.9, 133.5, 133.7, 136.4, 141.6, 149.7, 151.8, 153.0, 164.7, 166.2, 169.7.

Example 5R

9-(5-*O*-Benzoyl-3-*O*-benzyl-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (43, Fig. 4).

Nucleoside **42** (0.64 g, 1.06 mmol) was dissolved in a mixture of MeOH (16 mL) and saturated methanolic ammonia (16 mL). The mixture was stirred 0 °C for 1.5 h and then evaporated to dryness under reduced pressure. The residue was coevaporated with 96% EtOH (50 mL) and purified by column chromatography using a stepwise gradient of methanol (0-5%, v/v) in chloroform as eluent to afford 0.55 g of nucleoside **43** (92%) as a white solid

material. ^{13}C NMR ($CDCl_3$) δ 63.9, 72.7, 80.6, 82.3, 91.4, 123.2, 128.0, 128.1, 128.3, 128.6, 128.7, 129.1, 129.2, 129.8, 129.9, 133.1, 133.5, 133.6, 137.2, 141.4, 149.6, 151.1152.5, 164.7, 166.4.

Example 5S

9-(2-*O*-Acetyl-5-*O*-benzoyl-3-*O*-benzyl-α-L-ribofuranosyl)-6-*N*-benzoyladenine (**44**, Fig. 4).

Nucleoside **43** (0.45 g, 0.80 mmol) was dissolved in a mixture of anhydrous dichloromethane (20 mL) and anhydrous pyridine (4 mL). The stirred solution was cooled to -30 °C and trifluoromethanesulfonic anhydride (0.35 mL, 2.15 mmol) was added. After 1.5 h, the reaction mixture was allowed to warm to 0 °C and a saturated aqueous solution of sodium hydrogencarbonate (10 mL) was added followed by addition of dichloromethane (60 mL). The organic phase was separated, washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 70 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of anhydrous toluene (24 mL) and anhydrous dichloromethane (24 mL), and KOAc (0.39 g, 3.98 mmol) and 18-crown-6 (0.74 g, 2.79 mmol) were added at room temperature under stirring. The temperature was raised to 50 °C and stirring was continued for 16 h. After cooling to room temperature, dichloromethane (100 mL) was added, and the resulting mixture was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 50 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. he residue was purified by column chromatography using a stepwise gradient of methanol (0-5%, v/v) in chloroform as eluent to afford 0.38 g of nucleoside **44** (79%) as a white solid material.

Example 6

Oligomer synthesis and hybridization studies.

The oligomers were synthesized on an automated DNA synthesizer using the phosphoramidite approach (Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278). Building blocks **7, 31** and **32** were used for the synthesis of the α-L-RNA oligomers, *e.g.*, **11, 12** and **16-20** and **33-36.** The stepwise coupling yield for amidite **7** was approximately 98%, for amidite **31** approximately 94%, and for amidite **32** approximately 91% (20 min coupling time; 1*H*-tetrazole as activator) using procedures described in Rajwanshi, V. K.; Håkansson, A. E.; Dahl, B. M.; Wengel, J. *Chem. Commun.* **1999**, 1395. After detritylation, cleavage from the solid support and deacylation were affected using 40% aqueous methylamine (10 min, 55 °C). After cooling to –18 °C, the solid support was removed (centrifugation), washed [2 x 0.25 cm^3; EtOH:CH_3CN:H_2O (3:1:1, v/v/v)], and the combined liquid phase evaporated to dryness under reduced pressure. The residue was desilylated for 20 h at 55 °C or 20 h at RT using a method described by Wincott, F.; DiRenzo, A.; Schaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, D.; Gonzalez, C.; Scaringe, S.; Usman, N. *Nucleic Acids Res.* **1995**, *23*, 2677. Desilylation of the oligomers was preferentially performed at 55 °C as incomplete reaction was indicated for some oligomers when using milder desilylation conditions as revealed by MALDI-MS analysis.

The α-L-LNA phosphoramidite was prepared as out-lined in WO 00/66604 and the β-D-LNA phosphoramidite was prepared as outlined in WO 99/14226. The various natural nucleobases in-corporated into the oligomers was obtained as phosphoramidites from commercial suppliers.

Standard conditions of the synthesizer were used for incorporation of DNA monomers, whereas the incorporation of LNA or α-L-LNA monomers followed procedures described by Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455, Koshkin, A. A.; Singh, S. K.; Nielsen,

P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607, and Rajwanshi, V. K.; Håkansson, A. E.; Dahl, B. M.; Wengel, J. *Chem. Commun.* **1999**, 1395.

Analyses by capillary gel electrophoresis confirmed the purity of the novel α-L-RNA oligomers **11**, **12** and **16-20** as being >90%, MALDI-MS analyses confirmed their compositions (MALDI-MS ($[M-H]^-$ measured/$[M-H]^-$ calculated): **11** (2770/2769), **12** (2804/2801), **16** (3121/3121), **19** (3170/3169), **20** (3172/3169)).

The reference DNA oligomers **8** and **13**, the LNA oligomers **9** and **14**, and the α-L-LNA oligomers **10** and **15** have been prepared and studied previously (Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455, Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607, Rajwanshi, V. K.; Håkansson, A. E.; Kumar, R.; Wengel, J. *Chem. Commun.* **1999**, 2073, and Rajwanshi, V. K.; Håkansson, A. E.; Sørensen, M. D.; Pitsch, S.; Singh, S. K.; Kumar, R.; Nielsen, P.; Wengel, J. *Angew. Chem. Int. Ed.* **2000**, *39*, 1656).

Hybridization experiments (i.e., determination of T_m values) toward single-stranded DNA and RNA complements were conducted as described by Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607 or using conditions specified below for compound **33**.

For oligomers **8-12**, different variations of a 9-mer mixed-base sequence are studied. Introduction of three modified thymine LNA monomers (**9**) or three α-L-LNA monomers (**10**) significantly improves the thermal stability towards both DNA and RNA when compared to the results obtained with the corresponding DNA reference **8**. Both **9** and **10** display a weak RNA selectivity as witnessed by the slightly lower thermal stabilities of the duplexes involving the DNA complement. Results for the two α-L-RNA/DNA chimera **11** and **12**

are depicted in Table 1. Incorporation of one α-L-RNA nucleotide leads to unchanged (toward RNA) or reduced (toward DNA, ΔT_m = -4 °C) thermal stability when compared to the DNA reference **8**. When three α-L-RNA monomers are incorporated (oligomer **12**), hybridization towards both DNA and RNA is adversely influenced. However, hybridization is more adversely influenced towards DNA. The results for oligomers **11** and **12** indicate that the incorporation of one or more α-L-RNA monomers into an oligomer may impart selectivity towards hybridization with RNA targets into an oligomer.

Various combinations of the different monomers in a homothymine 10-mer context are evaluated in the second series of oligomers (oligomers **13-20**). The (almost) fully modified LNA and α-L-LNA oligomers **14** and **15**, respectively, display indeed very efficient hybridization towards both DNA and RNA. In contrast, a melting point was not obtained for the corresponding α-L-RNA **16** toward DNA or RNA. Exchange of four α-L-RNA monomers of **16** with LNA monomers gave the α-L-RNA/LNA chimera **17** and **18**. With four consecutive LNA monomers (**17**), no hybridization toward the DNA complement was detected. However, a T_m value of 27 °C toward the RNA complement was observed.

Table 1. Hybridization Studies

Oligomer No.	Oligomer Sequence [a,b]	Description	DNA Complement (T_m/C°) [c]	DNA [c] Complement (T_m/C°) [c]
8	GTGATATGC	DNA Reference	30	28
9	$GT^{L}GAT^{L}AT^{L}GC$	LNA/DNA	44	50

10	G($^{\alpha L}$T^{L})GA($^{\alpha L}$T^{L})A($^{\alpha L}$T^{L})GC	α-L-LNA/DNA	37	45
11	GTGA($^{\alpha L}$T)ATGC	α-L-RNA/DNA	26	28
12	G($^{\alpha L}$T)GA($^{\alpha L}$T)A($^{\alpha L}$T)GC	α-L-RNA/DNA	<5	12
13	$(T^{L})_{10}$	DNA Reference	20	19
14	$(T^{L})_{9}T$	LNA	80	71
15	$(^{\alpha L}T^{L})_{9}T$	α-L-LNA	63	66
16	$(^{\alpha L}T)_{9}T$	α-L-RNA	<5	<5
17	$(^{\alpha L}T)_{4}(T^{L})_{4}(^{\alpha L}T)T$	α-L-RNA/LNA	<5	27
18	$[(^{\alpha L}T)(T^{L})]_{4}(^{\alpha L}T)T$	α-LRNA/LNA	<5	<5
19	$(^{\alpha L}T)_{4}(^{\alpha L}T^{L})_{4}(^{\alpha L}T)T$	α-L-RNA/α-L-LNA	<5	29
20	$[(^{\alpha L}T)(^{\alpha L}T^{L})]_{4}(^{\alpha L}T)T$	α-L-RNA/α-L-LNA	<5	27

[a] All oligomers are depicted with the 5'-end positioned to the left. A = adenine monomer, C = cytosine monomer, G = guanine monomer, T = thymine monomer; A, C, G and T are DNA monomers, *i.e.*, 2'-deoxy-β-D-ribofuranosyl derivatives.

[b] T^{L} depicts a β-D-LNA monomer bearing a thymine nucleobase (thymin-1-yl), $^{\alpha L}T^{L}$ depicts a α-L-LNA monomer bearing a thymine nucleobase, and $^{\alpha L}T$ depicts α-L-LNA bearing a thymin-1-yl group.

[c] Melting temperatures (T_m values) towards complementary single-stranded DNA and RNA targets were obtained as the maximum of the first derivative of the melting curve (A_{260} *vs* temperature) in a salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two complements.

A comparable RNA selectivity has not been observed for longer homothymine sequences (*e.g.*, **14**) nor for partly or fully modified LNAs with mixed base compositions (see Oerum, H.; Jacobsen, M. H., Koch, T.; Vuust, J.; Borre, M. B. *Clin. Chem.* **1999**, *45*, 1898 and Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.

The α-L-RNA/LNA chimera **18** with alternating α-L-RNA and LNA monomers hybridizes neither with the DNA nor the RNA complement. The two α-L-RNA/α-L-LNA chimerae **19** and **20** containing four consecutive α-L-LNA monomers and alternating α-L-RNA and α-L-LNA monomers, respectively, display very similar binding properties. Thus, T_m values of 29 °C and 27 °C for **19** and **20**, respectively, were observed toward the RNA target, but no T_m values toward the DNA target.

In Table 2, results for 14-mer DNA, LNA/DNA,α-L-LNA/DNA, and α-L-RNA/DNA chimera as homothymine oligomers are depicted showing similar results as summarized in Table 1 above for the 9-mer series.

Furthermore, the following oligomer was studied:

5'-[($^{\alpha L}$U)($^{\alpha L}$A^{L})($^{\alpha L}$U)($^{\alpha L}$T^{L})($^{\alpha L}$U)($^{\alpha L}$A^{L})($^{\alpha L}$C)($^{\alpha L}$T^{L})($^{\alpha L}$U)($^{\alpha L}$T^{L})dC], (33)

where

$^{\alpha L}$T^{L} and $^{\alpha L}$A^{L} are α-L-LNA thymine and adenine monomers, respectively, and $^{\alpha L}$U and $^{\alpha L}$C are α-L-RNA uracil and cytosine monomers, respectively,

and dC is the DNA cytosine monomer incorporated in **33** for synthetic purpose (a dC support was used for synthesis).

The following DNA reference (**DNA-R**) and DNA (**DNA-T**) and RNA target (**RNA-T**) oligomers, the latter two oligomers containing a sequence complementary to the first ten monomers (the α-L-RNA/α-L-LNA segment) of **33**, were used for hybridization experiments:

DNA-R: 5'-d(TAT TTA CTT TC)

DNA-T: 5'-d(AAA GTA AAT A)

RNA-T: 5'-r(AAA GUA AAU A)

In a buffer of 1.0 M sodium chloride, 2.0 mM EDTA, 20 mM sodium phosphate, pH 7.0, the following melting temperatures (Tm values) were obtained:

Tm [**DNA-R:DNA-T**] = 23 ^{0}C

Tm [**DNA-R:RNA-T**] = 26 ^{0}C

Tm [**33:DNA-T**] = no Tm detectable above 5 ^{0}C

Tm [**33:RNA-T**] = 16 ^{0}C

These results indicate that α-L-RNA/α-L-LNA oligomers also in a mixed sequence context display RNA selective hybridization. Furthermore the results demonstrate that this is the case also in a high-salt hybridization buffer.

Example 7

Stability Studies of α-L-RNA/α-L-LNA Oligonucleotide towards Enzyme Degradation.

The stability of α-L-RNA/α-L-LNA chimera toward 3'-exonucleolytic degradation *in vitro* was evaluated using snake venom phosphodiesterase (SVPDE) using a procedure previously described by Rosemeyer, H.; Seela, F. *Helv. Chim. Acta* **1991**, *74*, 748 and Svendsen, M. L.; Wengel, J.; Dahl, O.; Kirpekar, F.; Roepstorff, P. *Tetrahedron* **1993**, *49*, 11341. During SVPDE digestion of unmodified oligonucleotides, e.g., the DNA reference **13**, the absorbance at 260 nm rapidly increases due to conversion into the nucleoside constituents. In contrast, the α-L-RNA/α-L-LNA chimera **19** and **20** are both very significantly stabilized toward degradation by SVPDE. These qualitative

experiments indicate that α-L-RNA/α-L-LNA chimera, like α-L-DNA (see Asseline, U.; Hau, J.-F.; Czernecki, S.; Le Diguarher, T.; Perlat, M.-C.; Valery, J.-M.; Thuong, N. T. *Nucleic Acids Res.* **1991**, *19*, 4067), are significantly protected toward 3'-exonucleolytic degradation.

Table 2. Hybridization Studies Using 14-mer Homothymine Sequences.

Oligomer Sequence [a,b]	Description	DNA Complement (T_m/C°) [c]	DNA [c] Complement (T_m/C°) [c]
T_{14}	DNA Reference	32	29
$T_5(T^L)_4T_5$	LNA/DNA	42	52
$T_5({}^{\alpha L}T^L)_4T_5$	α-L-LNA/DNA	36	46
$T_5({}^{\alpha L}T)_4T_5$	α-L-RNA/DNA	<5	11

[a] All oligomers are depicted with the 5'-end positioned to the left. T = thymine monomer

[b] T^L depicts a β-D-LNA monomer bearing a thymine nucleobase (thymin-1-yl), ${}^{\alpha L}T^L$ depicts a α-L-LNA monomer bearing a thymine nucleobase, and ${}^{\alpha L}T$ depicts a α-L-LNA bearing a thymin-1-yl group.

[c] Melting temperatures (T_m values) towards complementary single-stranded DNA and RNA targets were obtained as the maximum of the first derivative of the melting curve (A_{260} *vs* temperature) in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two complements.

Example 8

RNase H Cleavage

The complementary RNA oligonucleotide target was 5'-[^{32}P]-labelled with polynucleotide kinase and mixed with unlabelled RNA oligo. The α-L-RNA/DNA oligomer to be studied (**34-36**) was hybridized to the complementary RNA (4:1 ratio) in 20 mM Tris–HCl (pH 7.5), 100 mM KCl at 65 °C for 2 minutes followed by slow cooling to 37 °C. The RNase H digest

was performed in 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM KCl, 1 mM DTT by mixing the *E.coli* RNase H enzyme and the mixture of hybridized oligonucleotides and incubating at 37 °C. Aliquots of the reaction mixture were removed and stopped at 5 and 60 minutes after the initiation of the reaction and the reaction products were analyzed by electrophoresis on 20% acrylamide/urea gels followed by autoradiography.

RNA target sequence: 5'-r(AGG UCC AUA GAG AC)

DNA ref sequence: 5'-d(GTC TCT ATG GAC CT)

34: 5'-d(GTC TCT A($^{\alpha L}$U)G GAC CT) – Lanes marked "1" on the gel as shown in figure 1.

35: 5'-d(GTC ($^{\alpha L}$U)CT ATG GAC CT) – Lanes marked "2" on the gel as shown in figure 1.

36: 5'-d(G($^{\alpha L}$U)C TCT ATG GAC CT) – Lanes marked "3" on the gel as shown in figure 1.

Control (Co) is the complementary RNA oligonucleotide target that was 5'-[32P]-labelled with polynucleotide kinase. ($^{\alpha L}$U = α-L-RNA uracil monomer)

These data support the conclusion that the modified oligonucleotides **34-36** supports RNase H cleavage when hybridized to the complementary RNA sequence, but oligomer **34** is less efficiently cleaved than oligomers **35** and **36**. The RNA complement is cleaved most efficiently, but not exclusively, 3'to the α-L-RNA residue in the corresponding oligonucleotide. This suggests that RNAse H cleavage can take place in close proximity to an α-L-RNA monomer.

Other Embodiments

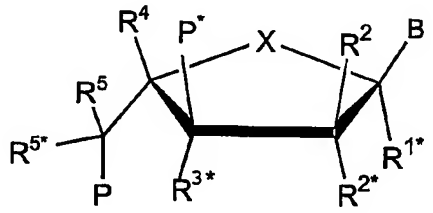
From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

What is claimed is:

CLAIMS

1. An oligonucleotide, comprising at least a first α-L-RNA monomer of the general formula:



I,

or a basic salt or acid addition salt thereof;

wherein X is -O-, -S-, -S(O)-, -$S(O)_2$-, -$N(R^{N*})$-, -$C(R^6R^{6*})$-, -O-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-O-, -S-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-S-,-$N(R^{N*})$-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-$N(R^{N*})$-, or -$C(R^6R^{6*})$-$C(R^7R^{7*})$-;

B is hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, optionally protected nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, or ligands;

P is a radical position for an internucleoside linkage to a second monomer or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R^5;

P* is an internucleoside linkage to a third monomer or a 3'-terminal group;

R^2 is F, Cl, Br, I, SR", SeH, SeR", $N(R^{N*})_2$, OH, a protected hydroxy group, SH, a protected mercapto group, an optionally substituted linear or branched C_{1-12}-alkoxy, or an optionally substituted linear or branched C_{2-12}-alkenyloxy;

each of the substituents R^{1*}, R^{2*}, R^{3*}, R^4, R^5, R^{5*}, R^6, R^{6*}, R^7, and R^{7*} is independently selected from hydrogen, optionally substituted linear or branched C_{1-12}-alkyl, optionally substituted linear or branched C_{2-12}-alkenyl, optionally substituted linear or branched C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, hetero-aryloxy, hydroxy protection group, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkylaminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkylcarbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, azido, sulfanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands,

where aryl and heteroaryl may be optionally substituted, where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a C_{1-5} alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)-, where R^N is selected from hydrogen and C_{1-4}-alkyl and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond, where R", when present, represents a C_{1-6}-alkyl or a phenyl group, and R^{N*}, when present, is selected from hydrogen and C_{1-4}-alkyl.

2. The oligonucleotide according to claim 1, wherein B designates a nucleobase selected from uracil-1-yl, thymin-1-yl, adenin-9-yl, guanin-9-yl, cytosin-1-yl, and 5-methyl-cytosin-1-yl.

3. The oligonucleotide according to claim 1 or 2, wherein X is selected from the group consisting of -O-, -S-, -S(O)-, $-S(O)_2-$, and $-N(R^{N*})-$.

4. The oligonucleotide according to claim 1, wherein X is -O-.

5. The oligonucleotide according to any of claims 1-4, wherein R^{1*}, R^{2*}, R^{3*}, R^4, R^5, or R^{5*} independently represent hydrogen, C_{1-4}-alkyl, or C_{1-4}-alkoxy.

6. The oligonucleotide according to any of the claims 1 to 5, wherein R^2 is hydroxy or a protected hydroxy group.

7. The oligonucleotide according to claim 6, wherein the protected hydroxy group is a linear or branched C_{1-6}-alkyl, or a silyl group substituted with one or more linear or branched C_{1-6}-alkyl groups.

8. The oligonucleotide according to any of the preceding claims, wherein R^2 is tert-butyldimethylsilyloxy.

9. The oligonucleotide according to any of the preceding claims, wherein P, when representing a 5'-terminal group, designates hydrogen, hydroxy, optionally substituted linear or branched C_{1-6}-alkyl, optionally substituted linear or branched C_{1-6}-alkoxy, optionally substituted linear or branched C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, monophosphate, diphosphate, triphosphate, or -W-A', wherein W is selected from -O-, -S-, and $-N(R^H)-$ where R^H is selected from hydrogen and C_{1-6}- alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

10. The oligonucleotide according to claim 9, wherein P is hydroxy or dimethoxytrityloxy.

11. The oligonucleotides according to any one of the preceding claims, wherein P*, when representing a 3'-terminal group, is selected from hydrogen, hydroxy, optionally substituted linear or branched C_{1-6}-alkoxy, optionally substituted linear or branched C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-4}- alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

12. The oligonucleotide according to any of the claims 1-8, wherein P or P* independently, when representing an internucleoside linkage to a first or second monomer, respectively, are selected from the group consisting of $-CH_2-CH_2-CH_2-$, $-CH_2-CO-CH_2-$, $-CH_2-CHOH-CH_2-$, $-O-CH_2-O-$, $-O-CH_2-CH_2-$, $-O-CH_2-CH=$, $-CH_2-CH_2-O-$, $-NR^H-CH_2-CH_2-$, $-CH_2-CH_2-NR^H-$, $-CH_2-NR^H-CH_2-$, $-O-CH_2-CH_2-NR^H-$, $-NR^H-CO-O-$, $-NR^H-CO-NR^H-$, $-NR^H-CS-NR^H-$, $-NR^H-C(=NR^H)-NR^H-$, $-NR^H-CO-CH_2-NR^H-$, $-O-CO-O-$, $-O-CO-CH_2-O-$, $-O-CH_2-CO-O-$, $-CH_2-CO-NR^H-$, $-O-CO-NR^H-$, $-NR^H-CO-CH_2-$, $-O-CH_2-CO-NR^H-$, $-O-CH_2-CH_2-NR^H-$, $-CH=N-O-$, $-CH_2-C(=NR^H)-O-$, $-CH_2-O-N=$, $-CH_2-O-NR^H-$, $-CO-NR^H-CH_2-$, $-CH_2-NR^H-O-$, $-CH_2-NR^H-CO-$, $-O-NR^H-CH_2-$, $-O-NR^H-$, $-O-CH_2-S-$, $-S-CH_2-O-$, $-CH_2-CH_2-S-$, $-O-CH_2-CH_2-S-$, $-S-CH_2-CH=$, $-S-CH_2-CH_2-$, $-O-CH_2-CH_2-O-$, $-S-CH_2-CH_2-O-$, $-S-CH_2-CH_2-S-$, $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, $-CH_2SO_2-CH_2-$, $-O-SO-O-$, $-O-S(O)_2-O-$, $-O-S(O)_2-CH_2-$, $-O-S(O)_2-NR^H-$,

$-NR^H-S(O)_2-CH_2-$, $-O-S(O)_2-CH_2-$, $-O-P(O)_2-O-$, $-O-P(O,S)-O-$, $-O-P(S)_2-O-$, $-S-P(O)_2-O-$, $-S-P(O,S)-O-$, $-S-P(S)_2-O-$, $-O-P(O)_2-S-$, $-O-P(O,S)-S-$, $-O-P(S)_2-S-$, $-S-P(O)_2-S-$, $-S-P(O,S)-S-$, $-S-P(S)_2-S-$, $-O-PO(R")-O-$, $-O-PO(OCH_3)-O-$, $-O-PO(BH_3)-O-$, $-O-PO(NHR^N)-O-$, $-O-P(O)_2-NR^H-$, $-NR^H-P(O)_2-O-$, $-O-P(O,NR^H)-O-$, and $O-Si(R")_2-O-$, wherein R^H represent hydrogen or a linear or branched C_{1-4}-alkyl group and R" represent a C_{1-6}-alkyl or phenyl group.

13. An oligomer according to claim 11, wherein any internucleoside linkage independently is selected from $-CH_2-CO-NR^H-$, $-CH_2-NR^H-O-$, $-S-CH_2-O-$, $-O-P(O)_2-O-$, $-O-P(O,S)-O-$, $-O-P(S)_2-O-$, $-NR^H-P(O)_2-O-$, $-O-P(O,NR^H)-O-$,
$-O-PO(R")-O-$, $-O-PO(CH_3)-O-$, and $-OPO(NHR^H)-O-$.

14. The oligonucleotide according to any of the claims 1 to 13, further comprising at least one LNA monomer.

15. The oligonucleotide according to claim 14, wherein the LNA monomer is an α-L-LNA monomer.

16. The oligonucleotide according to claim 14, wherein the LNA monomer is a β-D-LNA monomer.

17. The oligonucleotide according to any of the claims 14 to 16, wherein the at least one LNA is placed adjacent to the at least one α-L-RNA.

18. The oligonucleotide according to any of the claims 14 to 17, wherein group(s) of at least one LNA alternate(s) with group(s) of at least one α-L-RNA, optionally with intervening naturally occurring or synthetic nucleotides.

19. The oligonucleotide of claim 18, wherein each of the groups of LNA and α-L-RNA independently comprise 1 to 10 nucleotides.

20. The oligonucleotide according to any of the claims 18 or 19, comprising 1 to 50 alternations.

21. The oligonucleotide according to any of the claims 18 or 19, comprising 2 to 10 alternations.

22. The oligonucleotide according to any of the claims 14 to 21, comprising 1-100 LNA monomer(s), 1-100 α-L-RNA monomer(s), and 0-1000 nucleotides selected from natural occurring and synthetic nucleotides.

23. The oligonucleotide of claim 22, wherein the sum of the number of nucleotides in the oligonucleotide is at least 3.

24. The oligonucleotide according to any of the preceding claims, comprising 8 to 100 nucleotides.

25. The oligonucleotide according to any of the preceding claims, comprising at least 3 LNA monomers.

26. The oligonucleotide according to any of the preceding claims, comprising at least 3 α-L-RNA monomers.

27. The oligonucleotide according to claim 22, wherein the LNA monomer is a β-D-LNA.

28. The oligonucleotide according to claim 27, wherein the β-D-LNA is oxy-LNA.

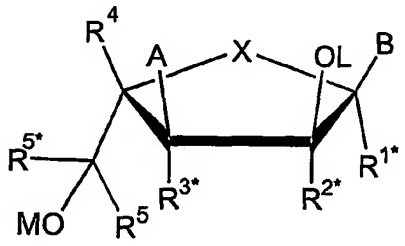
29. The oligonucleotide according to claim 28, comprising a continuous stretch of 2 to 30 oxy-LNA monomers.

30. The oligonucleotide according to any of the preceding claims, comprising a continuous, unbroken stretch of 3 to 30 monomers bearing a nucleobase of thymin-1-yl.

31. The oligonucleotide according to claim 30, wherein the monomers bearing the thymin-1-yl are of the α-L-RNA configuration exclusively.

32. A complex of an oligonucleotide as defined in any of the claims 1 to 31 and a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, and PNA.

33. A compound having the formula:



II,

or basic or acid addition salts thereof;

wherein X is selected from -O-, -S-, -S(O)-, -$S(O)_2$-, -$N(R^{N*})$-, -$C(R^6R^{6*})$-, -O-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-O-, -S-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-S-, -$N(R^{N*})$-$C(R^7R^{7*})$-, -$C(R^6R^{6*})$-$N(R^{N*})$-, and -$C(R^6R^{6*})$-$C(R^7R^{7*})$-, R^{N*}, when present, is selected from hydrogen and C_{1-4}-alkyl;

B is selected from hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, optionally protected nucleobases; DNA intercalators, photochemically active groups, thermo-chemically active groups, chelating groups, reporter groups, and ligands;

L is hydrogen or a hydroxy protection group;

M is hydrogen, a hyroxy protection group, or -P-(NR^8R^{8*})-R^9, wherein R^8 and R^{8*} independently are selected among linear or branched optionally substituted C_{1-6}-alkyl and C_{1-6}-alkenyl, and R^9 is a phosphate protection group;

A is hydroxy, protected hydroxy, or a phosphoramidite group of the general formula:

-O-P-(NR^8R^{8*})-R^9,

wherein R^8 and R^{8*} independently are selected among linear or branched optionally substituted C_{1-6}-alkyl and C_{1-6}-alkenyl, and R^9 is a phosphate protection group;

each of the substituents R^{1*}, R^{2*}, R^{3*},R^4, R^5, R^{5*}, R^6, R^{6*}, R^7, and R^{7*} is independently selected from hydrogen, optionally substituted linear or branched C_{1-12}-alkyl, optionally substituted linear or branched C_{2-12}-alkenyl, optionally substituted linear or branched C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkylaminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylaminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulfono, C_{1-6}-alkylsulfonyloxy, nitro, azido, sulfanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond.

34. The compound according to claim 33 wherein A is hydroxy or protected hydroxy and M is -P-(NR^8R^{8*})-R^9.

35. The compound according to claims 33 or 34, wherein X is selected from the group consisting of –O-, -S-, -S(O)-, -$S(O)_2$-, and -$N(R^{N*})$-.

36. The compound according to claim 35, wherein X is –O-.

37. The compound according to claims 33 to 36, wherein R^{1*}, R^{2*}, R^{3*}, R^4, R^5, or R^{5*} independently represent hydrogen, C_{1-4}-alkyl or C_{1-4}-alkoxy.

38. The compound according to claims 33 to 37, wherein the hydroxy protection group M is selected 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), 9-(9-phenyl)xanthenyl (pixyl), ethoxycarbonyloxy, phenylazo-phenyloxycarbonyloxy, tetrahydropyranyl (THP), 9-fluorenylmethoxycarbonyl (Fmoc), methoxytetrahydropyranyl (MTHP), trimethylsilyl (TMS), triisopropylsilyl (TIPS), tert-butyldimethylsilyl (TBDMS), triethylsilyl, phenyl-dimethylsilyl, benzyloxycarbonyl, 2-bromobenzyloxy-carbonyl, tert-butylether, methyl ether, acetals, acetyl, chloroacetyl, fluoroacetyl, isobutyroyl, pivaloyl, benzoyl, methoxymethyl (MOM), 2,6-dichlorobenzyl (2,6-Cl_2Bn).

39. The compound according to any of the claims 33 to 38, wherein L represents a protection group selected from 2'-O-triisopropylsilyloxymethyl (TOM) and 2'-O-t-butyldimethylsilyl (TBDMS).

40. The compound according to any of the claims 33 to 39, wherein the protected nucleobase is selected among cytosin-1-yl having the amino group at the 4-position protected, adenin-9-yl having the amino group at the 6-position protected, and gaunin-9-yl having the amino group at the 2-position protected by a group independently selected among acetyl (Ac), phenoxyacetyl (Pac), isopropylphenoxyacetyl (iPr-Pac), benzoyl (Bz), and dimethylformamidine (Dmf).

41. The compound according to any of the claims 33 to 40, wherein the optionally protected nucleobase is selected from the group consisting of 4-N-acetylcytosin-1-yl, 6-N-phenoxyacetyladenin-9-yl, 6-N-benzoyladenin-9-yl, 2-N-isopropylphenoxyacetylgaunin-9-yl, 2-N-dimethylform-amidinegaunin-9-yl, thymin-1-yl, 5-methyl-5-N-benzoyl-cytosine, 5-N-benzoylcytosine, and uracil-1-yl.

42. The compound according to any of the claims 33 to 41, wherein the protection group R^9 is selected from 2-cyanoethyloxy, 2,2,2- trichloro-1,1-dimethylethyloxy, p-nitrophenylethyloxy, methoxy, methylthio, and allyloxy.

43. A process for the production of an oligonucleotide comprising at least one α-L-RNA monomer, the process comprising the steps of

(a) providing a nucleoside unit attached to a solid support through a base labile bond, the nucleoside being protected on the 5'-position with an acid labile group,

(b) treating the solid support, having attached thereto a nucleoside according to step (a) or (g), with an acid to remove the 5'-protection group,

(c) adding a proton donating activator and a successive nucleoside monomer comprising at the 3'-position a phosphoramidite group and at the 5'-position an acid labile protection group,

(d) reacting the nucleoside attached to the solid support with the successive nucleoside phosphoramidite derivative to produce a phosphite triester linkage between the 5'-position of the nucleoside attached to the solid support and the 3'-position of the successive nucleoside monomer,

(e) optionally, capping unreacted 5'-hydroxy groups,

(f) adding an oxidizing agent to convert the phosphite triester group to a phosphodiester group,

(g) subjecting the oxidized product of step (f) to the steps (b) through (f) or

(h) adding a base to detach the support and protection groups, thus releasing the oligonucleotide, wherein at least one of the nucleoside monomers of step (c) are selected from the intermediates according to any of the claims 33 to 42.

44. The process according to claim 43, wherein one, more, or all hydroxy and exocyclic amino group(s) of the first nucleoside attached to the support and subsequently added nucleoside monomer(s) are protected with a base-labile group.

45. The process according to any of the claims 43 or 44, wherein the acid of step (b) is 2,2-dichloroacetic acid or 2,2,2-trichloroacetic acid.

46. The process according to any of the claims 43 to 45, wherein the reaction medium of step (b) is non-aqueous.

47. The process according to any of the claims 43 to 46, wherein the reaction medium is dichloromethane, trichloromethane, or toluene.

48. The process according to any of the claims 43 to 47, wherein the proton donating activator is 1H-tetrazole or a pyridinium salt.

49. The process according to any of the claims 43 to 48, wherein the capping of 5'-hydroxy groups in step (e) is accomplished by reacting the 5'-hydroxy groups with acetic anhydride.

50. The process according to claim 43, wherein the oxidizing agent is iodine.

51. A pharmaceutical composition comprising an oligonucleotide of any of the claims 1 to 32 and/or a compound of any one of claims 33 to 42, and a pharmaceutically acceptable carrier.

52. A method for the treatment, stabilization, or prevention of a disease in a mammal, said method comprising administering an oligonucleotide of any one of claims 1 to 32 or a composition of claim 51 to said mammal in an amount sufficient to treat, stablilize, or prevent said disease.

53. The method of claim 52, wherein said disease is selected from the group consisting of acute hepatic failure, autoimmune disorders, blood disorders, bone disorders, cancer, including bladder cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, cancer of the head and neck, lung cancer, metastatic cancer, liver cancer, leukemia, ovarian cancer, prostate cancer, renal cell carcinoma, sarcoma, and skin cancer, cardiovascular disease, gastrointestinal tract disorders, infectious disease, including HIV, inherited autosomal disease, mesothelioma, myopathies, neurological disorders, and neuropathy.

54. A method for the purification of RNA from a sample, said method comprising the steps of:

(a) contacting said sample with an oligonucleotide of any one of claims 1 to 42 that comprises a region with substantial complementarity to the corresponding region of an RNA of interest under conditions that allow said oligonucleotide to hybridize to said RNA of interest; and

(b) isolating said hydridized RNA.

55. The method of claim 54, wherein said isolation step comprises filtration, affinity chromatography, ion exchange chromatography, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, chromatofocusing, centrifugation, high pressure liquid chromatography, or dialysis.

56. The method of claim 54, wherein said oligonucleotide is bound to a support.

57. The method of claim 54, wherein said RNA of interest is mRNA.

58. A method of modulating the ability of a target oligonucleotide to act as a substrate for nucleic acid active enzymes, said method comprising hybridizing an oligonucleotide of any one of claims 1-13, 26, 30, and 31 to said target oligonucleotide.

59. The method of claim 58, wherein said enzyme is RNase H.

60. The method of claim 58, wherein said oligonucleotide consists of DNA monomers and one or more α-L-RNA monomer, and wherein said target oligonucleotide is a single-stranded or double-stranded RNA sequence.

61. The method of claim 60, wherein said hybridization results in a sequence-specific strand displacement.

62. The method of any of the claims 58 to 61, wherein said oligonucleotide is 5 -d(GTC TCT A($^{\alpha L}$U)G GAC CT), 5 -d(GTC ($^{\alpha L}$U)CT ATG GAC CT), or 5 -d(G($^{\alpha L}$U)C TCT ATG GAC CT).

63. A method of modulating the ability of a target oligonucleotide to act as a substrate for nucleic acid active enzymes, said method comprising hybridizing an oligonucleotide of any one of claims 1 to 32 to said target oligonucleotide.

64. The method of claim 63, wherein said enzyme is RNase H.

65. A method for binding an oligonucleotide to a target sequence in a dsRNA molecule, said method comprising contacting an oligonucleotide of claim 1 or 2 with a dsRNA molecule under conditions that allow said oligonucleotide to hybridize or bind to said dsRNA molecule by way of strand displacement or triple helix formation.

66. A method of increasing the resistance of an analyzing or purification system to degradation by one or more nucleic acid-active enzymes, said method comprising administering an oligonucleotide of any of the claims 14 to 32 to said system.

67. A method for catalyzing a biological or chemical reaction, said method comprising administering an oligonucleotide of any one of claims 1 to 32 to a reaction mixture in an amount sufficient to increase the rate of said reaction.

68. The method of claim 67, wherein said reaction is selected from the group consisting of self-modifiying biological reactions, Diels-Alder reactions, glycosidic bond formations, alkylations, acylations, and amide bond formations.

69. Use of an oligonucleotide according to any one of claims 1 to 32 for recovery of RNA from a mixture of biological materials.

70. Use of an oligonucleotide according to any one of claims 1 to 32 for selective recovery of mRNA from a mixture of biological materials.

71. Use of an oligonucleotide according to any one of claims 1 to 13 as a substrate for nucleic acid utilising enzymes.

72. Use according to claim 71, wherein the nucleic acid utilising enzyme is RNase H.

73. Use of an oligonucleotide according to any one of claims 1 to 13 wherein all nucleotides except the α-L-RNA monomer(s) are DNA monomers for binding to a single stranded or doubled stranded RNA target sequence.

74. Use according claim 73,. wherein said binding result in a sequence specific strand displacement.

75. Use according to any one of claims 69 to 74 wherein said oligonucleotide is selected from oligonucleotides 34 to 36 of Example 8 herein.

76. Use of a combination of α-L-LNA and α-L-RNA in an oligonucleotide according to any of the claims 14 to 32 to modulate the ability of the oligonucleotide to act as a substrate for nucleic acid active enzymes.

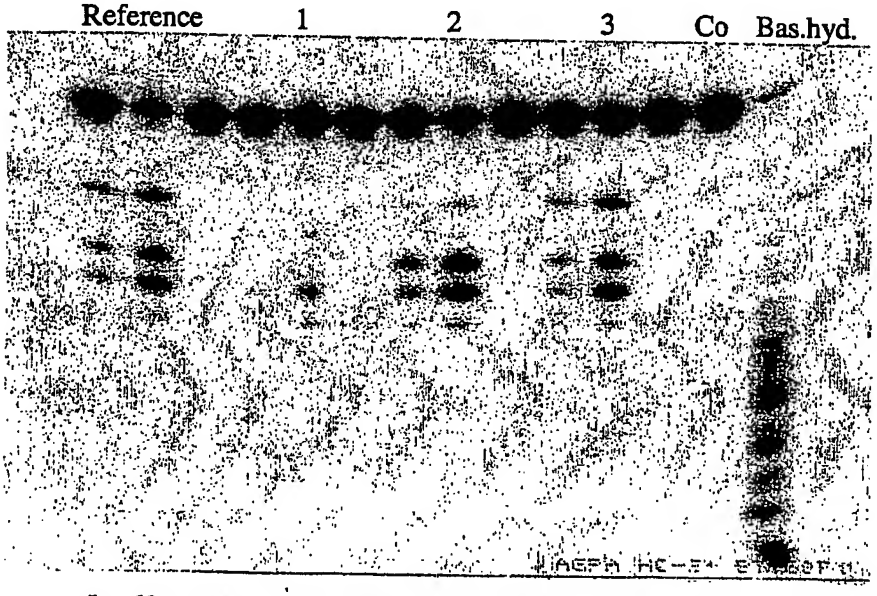
77. Use according to claim 76, wherein the nucleic acid active enzyme is RNase H.

78. Use of an oligonucleotide according to any of the claims 1 to 32, for binding to a target sequence in a dsRNA molecule by way of strand displacement or triple helix formation.

79. Use of an oligonucleotide according to any of the claims 14 to 32 to impart into an analyzing or purification system resistance to degradation by nucleic acid active enzymes.

80. Use of the oligonucleotide according to any of the claims 1 to 32, as a catalyst for hydrolysis of phosphodiester linkages or bonds.

Figure 1



* incubated minus enzyme

Figure 2

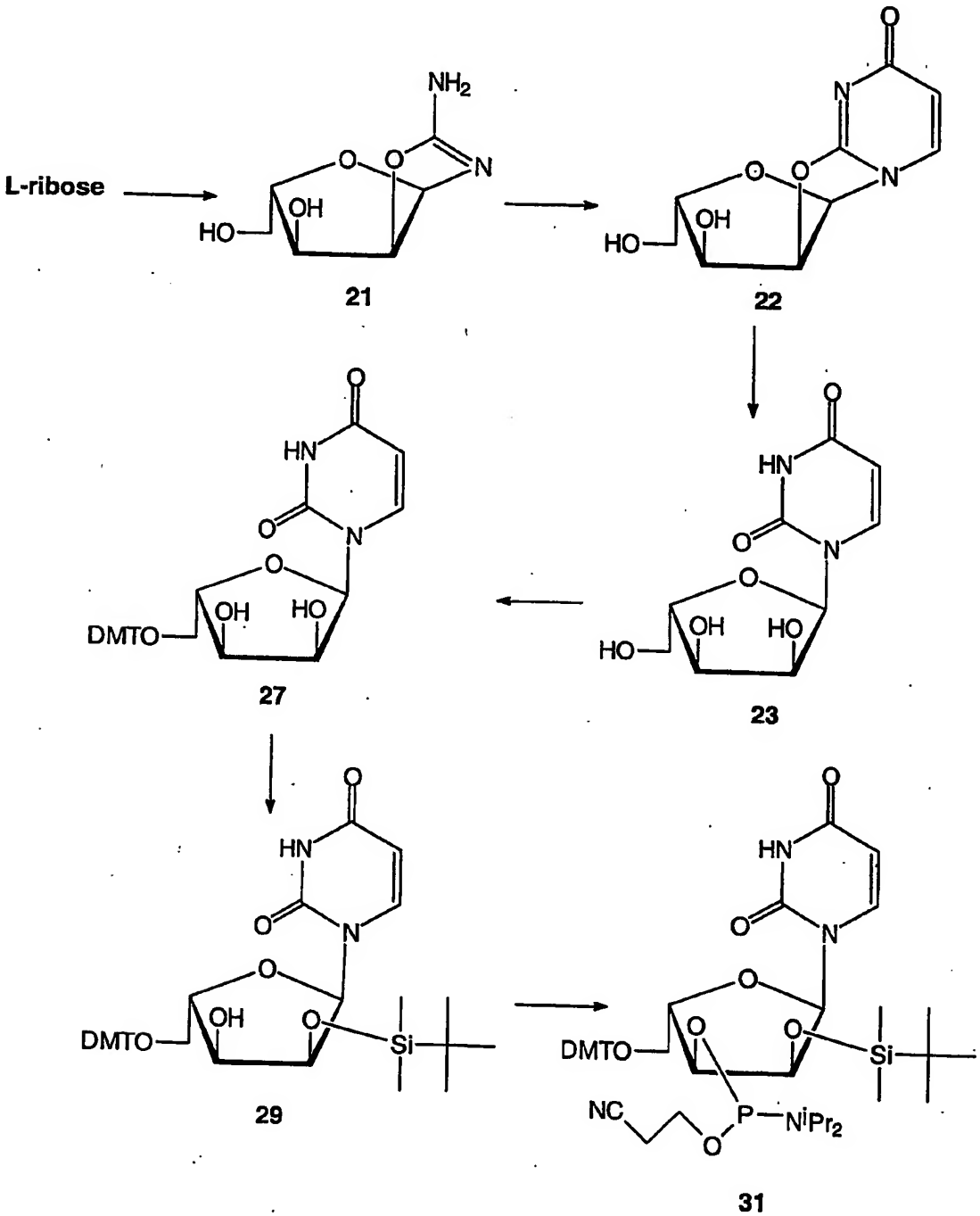
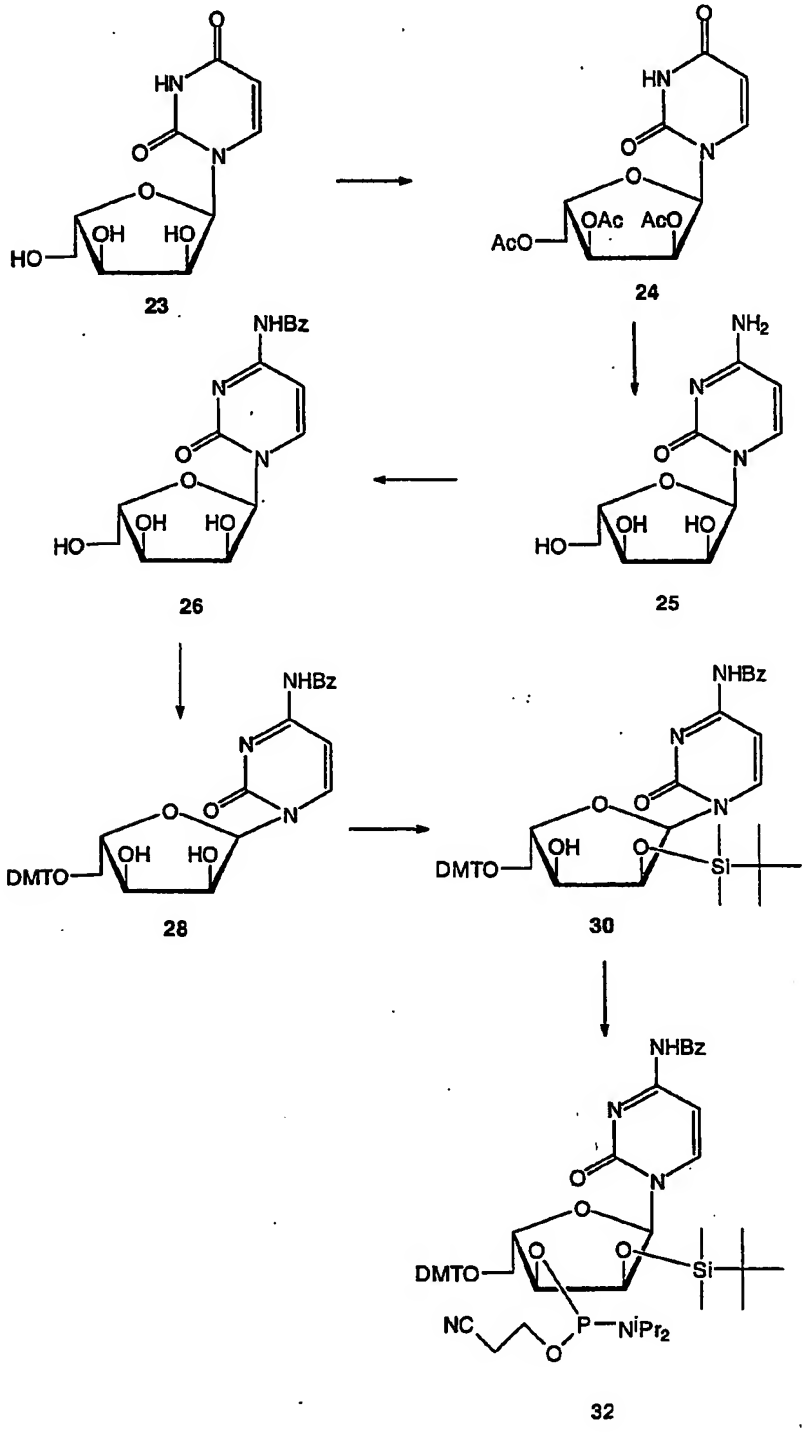


Figure 3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HO
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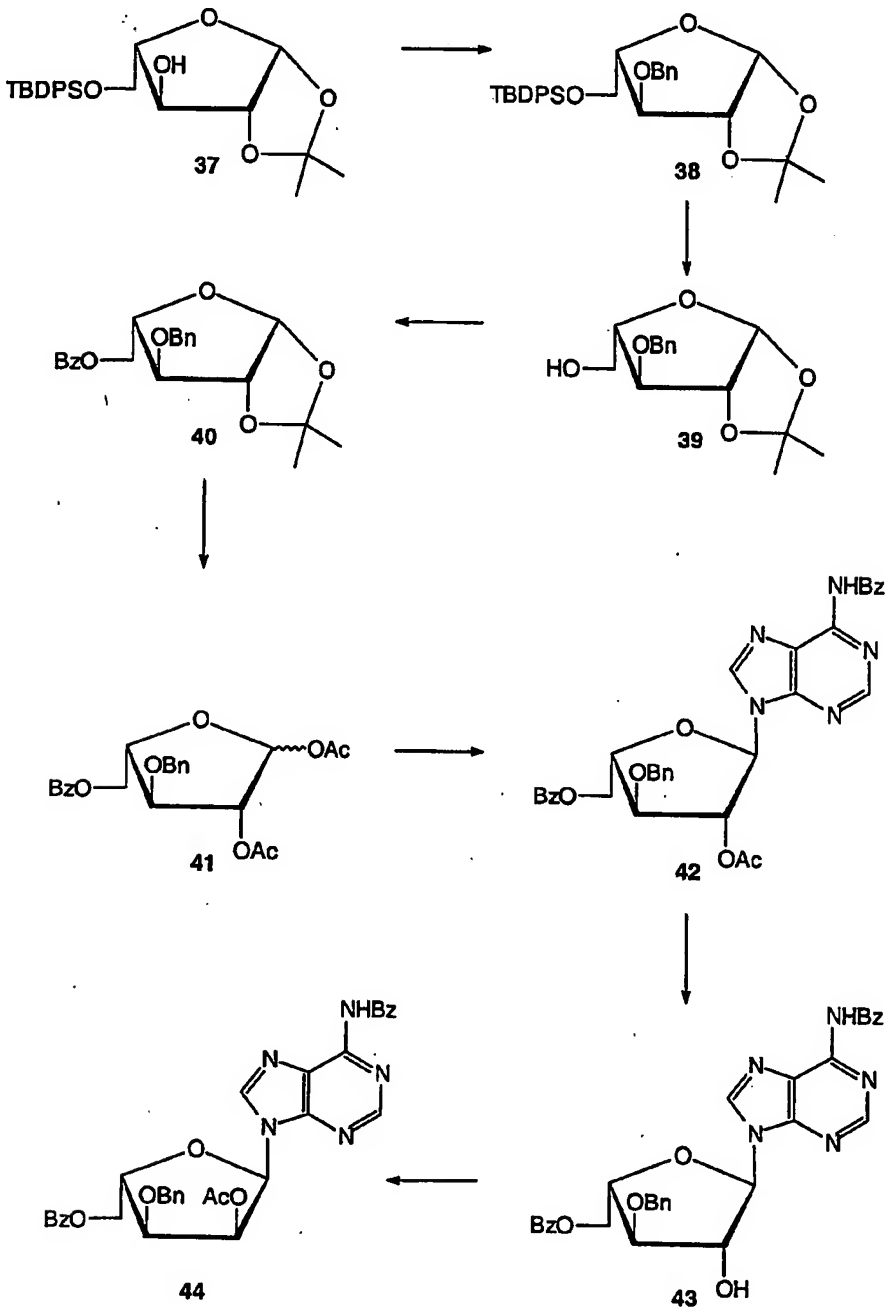
NC
P
NiPr2
32

Figure 4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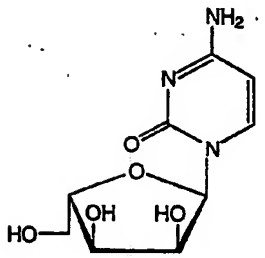

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Fig. 5

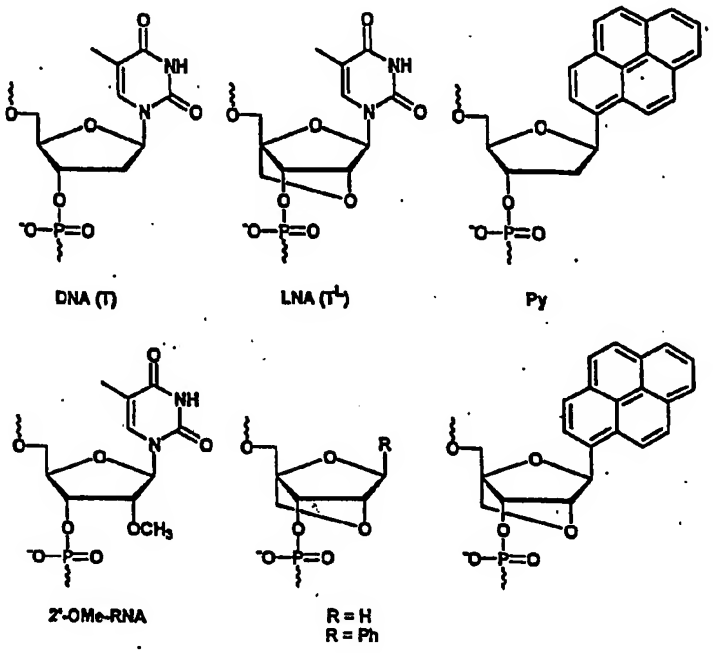

Figure 6